Brief Definitive Report

The Notch Ligand Jagged-1 Represents a Novel Growth Factor of Human Hematopoietic Stem Cells

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Abstract

The Notch ligand, Jagged-1, plays an essential role in tissue formation during embryonic development of primitive organisms. However, little is known regarding the role of Jagged-1 in the regulation of tissue-specific stem cells or its function in humans. Here, we show that uncommitted human hematopoietic cells and cells that comprise the putative blood stem cell microenvironment express Jagged-1 and the Notch receptors. Addition of a soluble form of human Jagged-1 to cultures of purified primitive human blood cells had modest effects in augmenting cytokine-induced proliferation of progenitors. However, intravenous transplantation of cultured cells into immunodeficient mice revealed that human (h)Jagged-1 induces the survival and expansion of human stem cells capable of pluripotent repopulating capacity. Our findings demonstrate that hJagged-1 represents a novel growth factor of human stem cells, thereby providing an opportunity for the clinical utility of Notch ligands in the expansion of primitive cells capable of hematopoietic reconstitution.

Key words: hematopoiesis • Jagged-1 • Notch • stem cells • development

Introduction

The evolutionarily conserved Notch signaling pathway has been shown to play a critical role in the development and patterning of a wide variety of organisms including worms, flies, and mice (1). Instructive Notch signals are mediated by cell–cell interactions between Notch receptor and Notch ligands that control cell fate decisions. Although the definite relationship between Notch signaling and biological outcome is dependent on the specific cell type, growth factor environment, and species, it is clear that Notch activation is capable of inhibiting differentiation and progression of precursor populations to more developmentally restricted cells (2, 3). In addition to pluripotency, many of these precursors are capable of immense proliferative capacity, suggesting that Notch activation may be intricately related to the control of differentiation and proliferation of stem cells (4). Although fundamental influences of Notch signaling in developmental processes may be pertinent to medical applications (5), the specific role of ligand-induced Notch activation in primary human stem cells is currently unknown.

Notch receptors interact with a family of transmembrane proteins that serve as ligands containing a highly conserved Delta-Serrate-Lag-2 (DSL) domain in the extracellular region. Activation of Notch signaling is mediated through interactions between the DSL domain and specific epidermal growth factor (EGF) repeats located in the extracellular portion of the Notch receptor (6). The Drosophila Notch ligand, Serrate, is a family member of Notch ligands cloned from invertebrates (7, 8). Jagged-1, the mammalian homologue of Serrate, has been shown to be a potent activator of the Notch signaling pathway in a variety of cell types, mediating signals via cellular interactions with adjacent Notch-expressing cells (9). Here, using hematopoietic development as a well characterized model system for primary human cells, we investigated the potential role of Jagged-1–induced Notch signaling in humans (10).

Ex vivo culture conditions originally optimized using surrogate in vivo models to detect human hematopoietic stem cell function in immunodeficient recipients have recently led to enhanced hematopoietic reconstitution and

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retroviral gene transfer of repopulating stem cells in clinical trials (11, 12). However, although these ex vivo culture conditions provide a therapeutic benefit, the magnitude of the expansion and gene transfer efficiency is insufficient for the treatment of a wider variety of patient pathologies. Therefore, the success of ex vivo expansion conditions still awaits the identification of factors capable of inducing proliferation of rare stem cells. In this study, production of a soluble form of human (h)Jagged-1 provided a means to functionally evaluate the role of hJagged-1 in ex vivo cultures of highly purified human hematopoietic cells. Although addition of hJagged-1 had modest effects in enhancing cytokine-induced proliferation of primitive progenitors detected in vitro, intravenous transplantation of cultured cells into immunodeficient nonobese diabetic (NOD)/SCID mice revealed that hJagged-1 is capable of maintaining the survival and expanding human stem cells capable of pluripotent reconstituting capacity. Unlike hematopoietic cytokines, this unique function of hJagged-1 provides an opportunity for the optimization for clinical protocols aimed at ex vivo expansion and gene transfer of human stem cells.

Materials and Methods

Expression of Human Notch 1 and 2 and Jagged-1. Reverse transcription (RT)-PCR reactions were performed on cDNA using primer sequences as follows: Notch 1, forward 5′-GATGCCAACATCCAGGACAACATGGG-3′ and reverse 5′-GGCAGGCGGTCCATATGATCCGTGAT-3′; Notch 2, forward 5′-ACATCATCACAGACTTGGTC-3′ and reverse 5′-CATTATTGACAGCAGCTGCC-3′; and Jagged-1, forward 5′-GATCCTGTCCATGCAGAACG-3′ and reverse 5′-GGATCTGATACTCAAAGTGG-3′. PCR products were sequenced to verify specificity of amplified DNA.

Preparation of hJagged-1 Protein. hJagged-1 cDNA was isolated by plaque hybridization from human placenta and fetal brain cDNA libraries (CLONTECH Laboratories, Inc.). Sequences were determined and verified using an ABI DNA sequencer (model 375S; PerkinElmer). Partial cDNA encoding the entire extracellular domain of Jagged-1 was fused in frame to a sequence of human IgG_1 or FLAG octapeptide. Jagged-1 gene fusions were inserted into the expression vector pcDNA3 (Invitrogen) or pMKITneo (provided by Dr. K. Maruyama, Tokyo Medical and Dental University, Tokyo, Japan) and electroporated into Chinese hamster ovary cells with subsequent G418 selection. Expression of soluble Jagged-1 protein was verified with anti–human IgG_1 (Amersham Pharmacia Biotech) or anti-FLAG (M2; Eastman Kodak Co.) antibody. Both human IgG_1 and FLAG chimera proteins were purified from conditioned media by affinity chromatography according to methods previously described (13).

Cell Purification. Human cord blood (CB) mononuclear cells were isolated and enriched for primitive uncommitted cells (lineage [Lin]$^-$) with $CD34^+CD38^-Lin^-$ cell populations subsequently isolated by sorting, as previously described (14). Mature populations of myeloid, T, and B cells were isolated using human-specific CD33, CD3, and CD19, respectively.

Treatment of Cells with hJagged-1. Purified $CD34^+CD38^-Lin^-$ CB cells were cultured in serum-free media containing cytokines designed to sustain human repopulating stem cells, as previously described (14). Cells were incubated for indicated periods at 37°C and 5% CO_2 in the presence of IgG_1 (control treated) or 10 μg/ml hJagged-1–IgG_1. Recombinant IgG_1 proteins were added to cultures treated with cytokine cocktail alone, in the absence of hJagged-1, to control for nonspecific effects of IgG_1 protein. Recombinant IgG_1 proteins had no effect compared with hematopoietic cytokine cocktail alone. Shorter term cultures analyzed at 4–12 d were done independently of cultures analyzed at 15–25 d.

Clonogenic Progenitor Assays. Human clonogenic progenitor assays were performed by plating equal numbers of Jagged-1 or control treated cells using previously defined conditions (14) and scoring after 10–14 d.

Transplantation and Analysis of NOD/SCID Mice. Cultured cells were transplanted via tail vein injection into irradiated NOD/SCID mice according to standard protocols along with 100,000 irradiated $CD34^-Lin^+$ cells (14). Mice were killed 6–8 wk after transplantation, and murine bone marrow (BM) cells were collected for analysis. Genomic DNA was extracted from the BM cells of transplanted mice and 1–2 μg of DNA that was EcoRI digested and probed using a human-specific chromosome 17 α satellite probe (p17H8) as shown previously (14). Quantitation was performed by comparison to standards of known murine and human DNA mixtures. In addition, BM cells were stained with fluorochrome-conjugated antibodies specific to human CD45 (a pan leukocyte marker) and CD38 (Becton Dickinson) and analyzed by flow cytometry using a FACSCalibur™ and CELLQuest™ software (Becton Dickinson). Multilineage analyses of BM cells from engrafted animals were gated to analyze human cells only in combination with either CD20–FITC and CD19–PE, CD33–FITC (Becton Dickinson) and CD15–PE (Immunotech), or CD34–FITC and CD38–PE (Becton Dickinson) mAbs.

Results

Expression of hJagged-1 and Notch Receptors among Human Hematopoietic Cells and Their Microenvironment. Using in vitro assays in mouse or human cell lines, investigators have suggested that Notch signals may influence the growth of hematopoietic progenitors detected in vitro (15–17). In addition, a bulk population of primitive $CD34^+$ human blood cells isolated from adult BM have been shown to express the Notch 1 receptor (18) and have been thought to respond to Notch signal activation via interactions with ligands expressed by adjacent cells. Adult stromal or human umbilical vein endothelial cells (HUVECs) constitute the microenvironment of hematopoietic stem cells found in BM and CB, respectively. Here, primary BM stromal cells and HUVECs were shown to express hNotch 1 and hNotch 2 receptors in addition to Jagged-1, indicating that these cells are capable of both responding to and eliciting Notch signaling (Fig. 1 A). To further investigate the interplay between Notch and Jagged-1 in human hematopoietic cells, primary human hematopoietic tissue from full gestational age CB was fractionated by cytometric cell sorting into subsets of various lineages representing myeloid ($CD33^+$), T ($CD3^+$), and B ($CD19^+$) lymphoid cells using lineage-specific markers (19). In addition, extremely rare, primitive cells (<0.05% of mononuclear blood cells) depleted of lineage-restricted cells (Lin^-) expressing the stem cell marker CD34 were separated into $CD34^+CD38^+Lin^-$

or candidate human hematopoietic $CD34^+CD38^-Lin^-$ stem cell subfractions (19, 20). The level of purification of these subsets was verified by FACS® reanalysis and demonstrated >97% purity (Fig. 1 B). Notch 1 and Notch 2 receptors were shown to be expressed throughout human hematopoietic maturation, indicating that human blood cells are capable of responding to Notch ligands (Fig. 1 C). Surprisingly, the Notch ligand Jagged-1 was also expressed by both primitive and mature human hematopoietic cells (Fig. 1 C). Therefore, in addition to responding to Notch-activating signals from cognate stromal or endothelial microenvironments, primitive human blood cells expressing Jagged-1 may be capable of inducing Notch signaling among surrounding cells. Our data reveals that Notch receptor and ligand interactions within the human hematopoietic system are more complex than previously appreciated (21) and may not only be restricted to Notch activation of hematopoietic cells by stromal environments but may include signaling among mature and primitive hematopoietic cells alike.

Soluble hJagged-1 Is Capable of Specific Interaction with Primitive Human Hematopoietic Cells. Based on our expression studies, we sought to further define the functional role of Jagged-1 in human hematopoietic stem cells using a soluble form of hJagged-1. hJagged-1 cDNA was expressed in transfected producer cell lines as a chimeric protein of either human IgG_1 or FLAG protein sequences and purified from supernatants by affinity chromatography (13). Purified soluble hJagged-1 was separated on SDS-PAGE and visualized by Coomassie blue staining (Fig. 2 A, i). Purity and integrity of soluble hJagged-1 was verified by Western blotting using anti–human IgG_1 or anti-FLAG antibodies (Fig. 2 A, ii); a single protein product of the expected molecular size was produced. In addition, purified Jagged-1 protein product was shown to bind specifically to the Notch receptor overexpressed in transfected COS cell lines (data not shown).

To determine whether primary human uncommitted hematopoietic cells (Lin^-) were capable of binding soluble Jagged-1, human Lin^- cell fractions were treated with hJagged-1–FLAG protein followed by a complex of mouse anti-FLAG fluorochrome-conjugated secondary antibody and analyzed by flow cytometry. hJagged-1 was shown to bind to primitive human hematopoietic cells (Fig. 2 B). Specificity of hJagged-1 binding was demonstrated by competing hJagged-1–FLAG cellular interaction by pre-

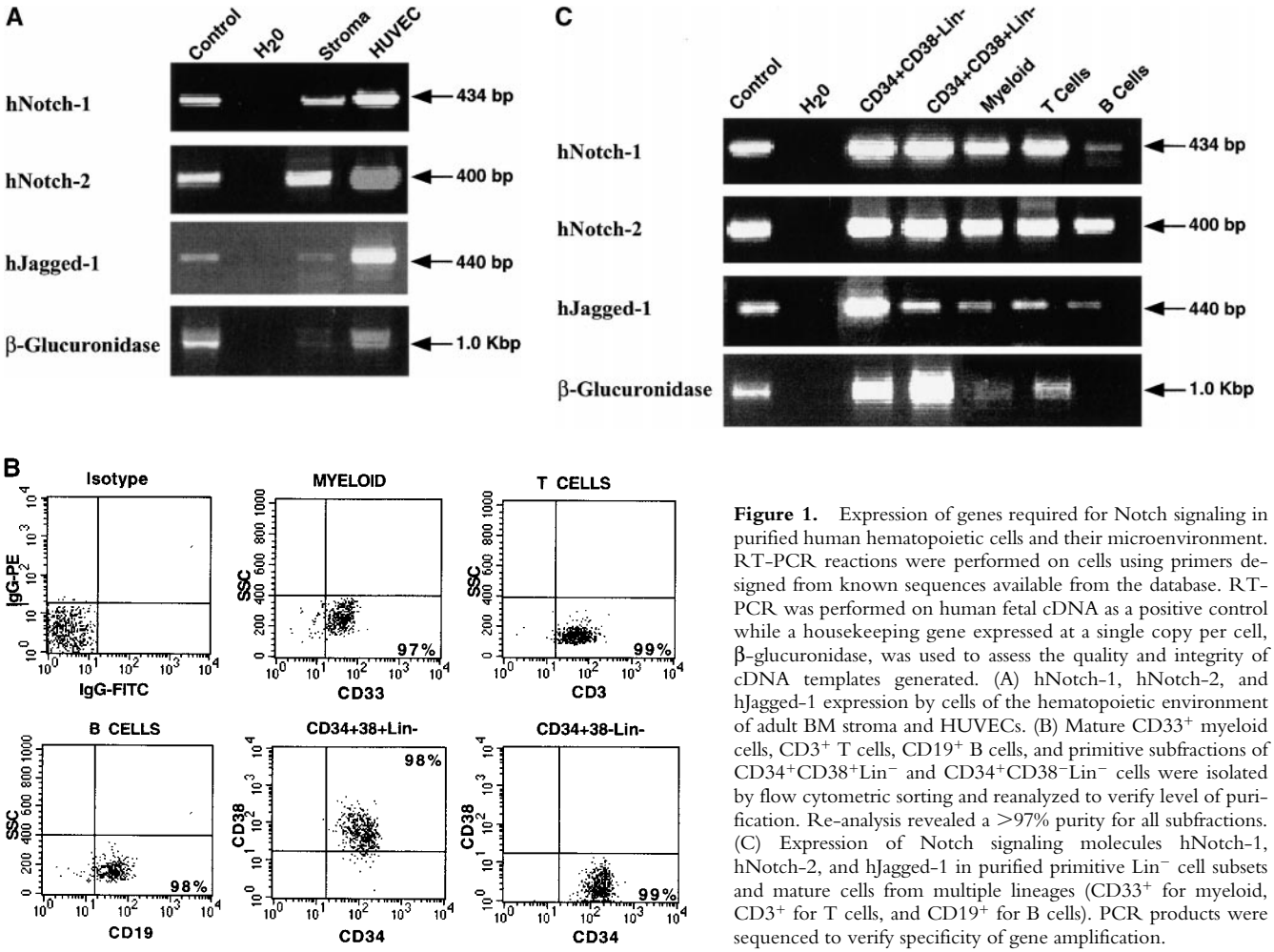

Figure 1. Expression of genes required for Notch signaling in purified human hematopoietic cells and their microenvironment. RT-PCR reactions were performed on cells using primers designed from known sequences available from the database. RT-PCR was performed on human fetal cDNA as a positive control while a housekeeping gene expressed at a single copy per cell, β-glucuronidase, was used to assess the quality and integrity of cDNA templates generated. (A) hNotch-1, hNotch-2, and hJagged-1 expression by cells of the hematopoietic environment of adult BM stroma and HUVECs. (B) Mature $CD33^+$ myeloid cells, $CD3^+$ T cells, $CD19^+$ B cells, and primitive subfractions of $CD34^+CD38^+Lin^-$ and $CD34^+CD38^-Lin^-$ cells were isolated by flow cytometric sorting and reanalyzed to verify level of purification. Re-analysis revealed a >97% purity for all subfractions. (C) Expression of Notch signaling molecules hNotch-1, hNotch-2, and hJagged-1 in purified primitive Lin^- cell subsets and mature cells from multiple lineages ($CD33^+$ for myeloid, $CD3^+$ for T cells, and $CD19^+$ for B cells). PCR products were sequenced to verify specificity of gene amplification.

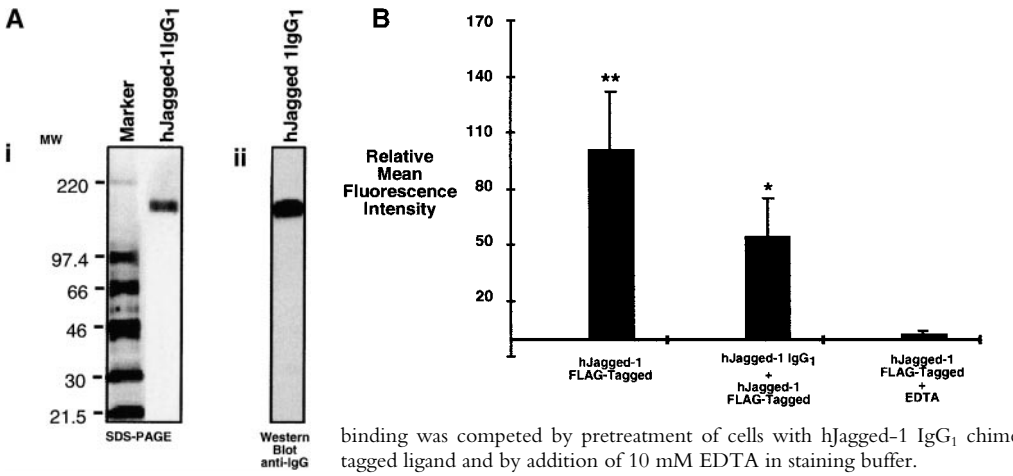

Figure 2. Characterization of soluble hJagged-1. (A) Analysis of Jagged-1 by Coomassie blue–stained SDS-PAGE (i) and Western blot analysis of purified hJagged-1 IgG_1 chimera using secondary, goat anti–human IgG_1 antibody (ii). MW is expressed in kilodaltons. (B) Specific binding of hJagged-1–FLAG chimera by human hematopoietic Lin^- cells. Results are expressed as mean fluorescence intensity ± SEM compared with controls ($n = 5$). *Significant difference $P < 0.05$. * and ** are significantly different from each other, $P < 0.01$. hJagged-1–FLAG binding was competed by pretreatment of cells with hJagged-1 IgG_1 chimera before treating with the FLAG-tagged ligand and by addition of 10 mM EDTA in staining buffer.

treating cells with hJagged-1–IgG_1 (Fig. 2 B) or fully abolishing hJagged-1–FLAG binding capacity with the addition of 10 mM EDTA, which prevents Ca^{2+}-mediated Jagged–Notch interactions of DSL–EGF domains (22) (Fig. 2 B). EDTA or hJagged-1–IgG_1 pretreatment had no effect on the binding efficiency of other cell surface–targeted, fluorochrome-conjugated antibodies detected by flow cytometry such as human CD34 (data not shown). Our results illustrate that primitive human hematopoietic cells expressing the Notch receptor are capable of Jagged-1 ligand interactions, thus providing a novel system in which to investigate the biological outcome of Jagged-1–induced Notch signaling in humans.

hJagged-1 Induces Proliferation of Human Hematopoietic Progenitors. To characterize the role of Jagged-1 in human blood formation and development, highly purified $CD34^+CD38^-Lin^-$ human stem cells (purity >98%, Fig. 1 B) were isolated and cultured in optimized serum-free conditions (23–25). Cells cultured with hematopoietic cytokines were compared with those treated with soluble hJagged-1. Recombinant IgG_1 protein was added to cultures treated with cytokine cocktail alone in the absence of hJagged-1 (control treated) to control for nonspecific effects of IgG_1. Addition of recombinant IgG_1 protein at equivalent concentrations had no effect when compared conditions with hematopoietic cytokines cocktail alone. Cultures were harvested at indicated times and examined for changes in total cell proliferation, expansion of primitive $CD34^+CD38^-$ subsets, and multilineage progenitor function detected in vitro (Fig. 3). hJagged-1 treatment (10 μg/ml) was able to expand the total number of cells in short term cultures after 9 d. However, this modest effect was lost after prolonged long term cultures beyond 15 d (Fig. 3 A). Although hJagged-1 had little effect on the bulk culture of cells, hJagged-1 was able to demonstrate more noticeable expansion of total number of more primitive $CD34^+CD38^-$ subsets in the culture between 9 and 25 d, as compared with control treated cultures in the absence of hJagged-1 (Fig. 3 B). In addition, the frequency of $CD34^+CD38^-$ cells in Jagged-1 treated cultures was consistently higher throughout all periods of culture as compared with cultures treated with hematopoietic cytokines alone (Fig. 3 B). The expansion of the $CD34^+CD38^-$ subsets (and total $CD34^+$ cells; data not shown) in response to Jagged-1 suggests that Jagged-1 is capable of modestly modulating phenotypically primitive human blood cells. In addition to proliferative and phenotypic analysis, functional assays for hematopoietic progenitors was performed after 4–25 d of hJagged-1 treatment and compared with control treated cultures (Fig. 3 C). Although hJagged-1 treatment did not increase clonogenic progenitors during early periods of ex vivo culture (<6 d), cells exposed to hJagged-1 for extended periods enhanced the expansion of progenitors of multiple myeloid lineages (Fig. 3 C). Treatment with hJagged-1 did not affect the developmental program of progenitors, as the composition of lineage-restricted progenitors (CFU type) did not differ between Jagged-1–treated and control treated cultures (data not shown). Taken together, our analysis of primary human blood cells treated with hJagged-1 illustrates that Jagged-1 enhances cytokine-induced proliferation of rare primitive subsets of human hematopoietic cells without altering lineage development.

hJagged-1 Represents a Hematopoietic Growth Factor Capable of Expanding Pluripotent Human Repopulating Cells. The response of primitive human blood cells to Jagged-1 in vitro suggests that Jagged-1 may play a role in modulating rarer pluripotent hematopoietic stem cells capable of repopulation. To explore the potential effects of Jagged-1 in regulating primitive human repopulating cells, purified cells cultured with or without hJagged-1 were transplanted into immunodeficient NOD/SCID mice (24, 26, 27). To quantitatively determine the role of hJagged-1 on the number of human blood stem cells, individual wells were seeded with highly purified $CD34^+CD38^-Lin^-$ cells; cultures were treated for periods of 4 to 15 d and then harvested for intravenous transplantation into NOD/SCID mice. The BM of transplanted animals was analyzed 8 wk after transplant by flow cytometry and Southern blot analysis to determine the presence or absence of human repopulating cells. Wells were seeded with 500–2,500 $CD34^+$ $CD38^-Lin^-$ cells containing approximately two to three

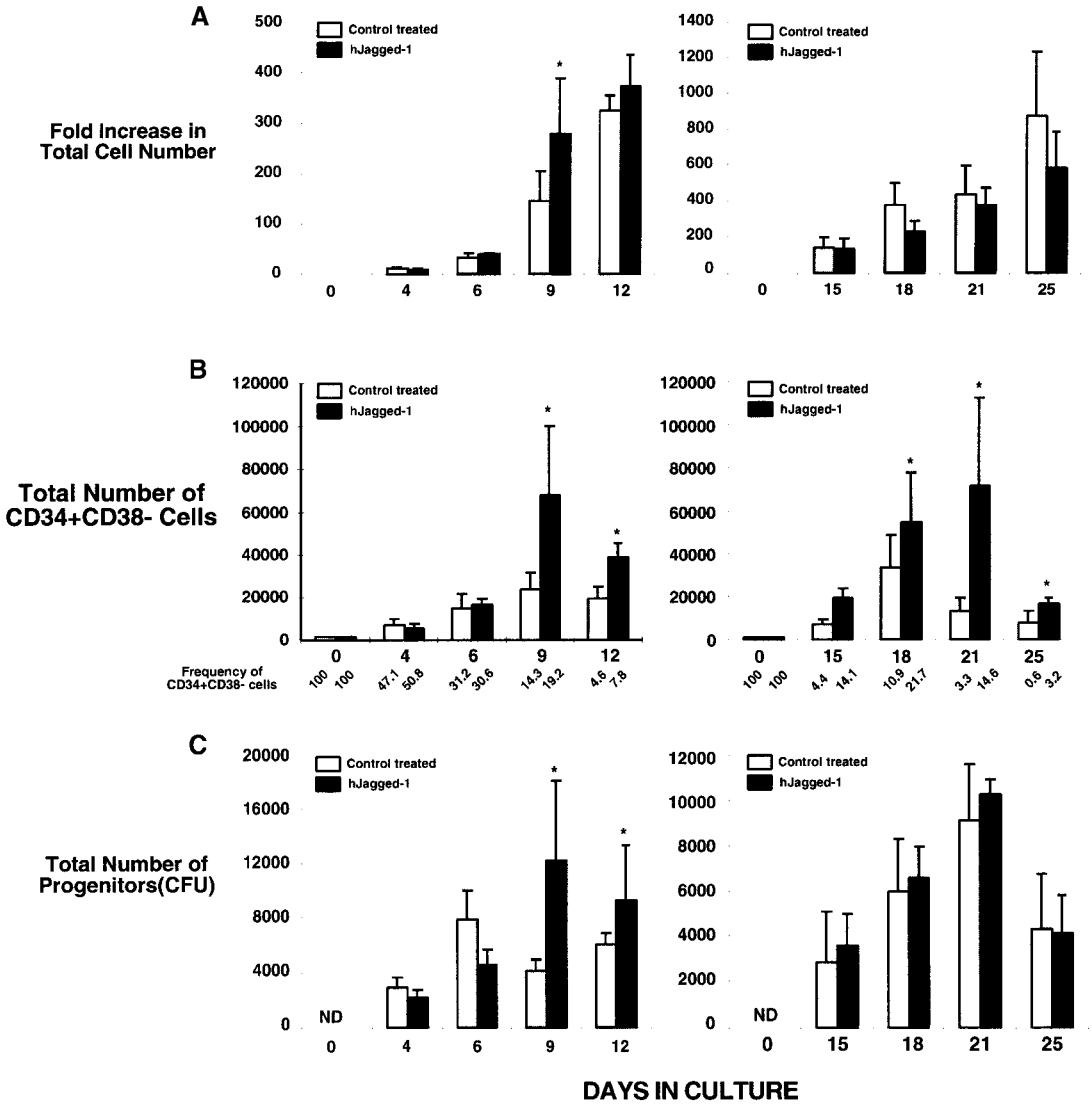

Figure 3. Functional in vitro analysis of primary human $CD34^+CD38^-Lin^-$ cells cultured in serum-free conditions containing hJagged-1. Highly purified $CD34^+CD38^-Lin^-$ cells were isolated (purity >99%, Fig. 1 B) and seeded in 96-well plates containing serum-free medium and hematopoietic cytokines in the presence of 10 μg/ml of soluble hJagged-1 or control recombinant IgG_1. Cells were harvested at the indicated times, counted, phenotyped, and seeded into methylcellulose for progenitor cell assays. (A) The fold increase in total cell number relative to cells seeded on day 0. Cells from individual wells were counted, and the mean fold increase in absolute cell number was determined. Mean values ± SEM are shown. Viability of control treated and hJagged-1–treated cells was consistently >97%, indicating that increases in cell number and culture expansion are due to proliferation as opposed to differential survival (data not shown). (B) Changes in the total number of primitive $CD34^+CD38^-$ cells in culture. The total number of $CD34^+CD38^-$ cells was calculated using the total cell count from each well, and the frequencies of $CD34^+CD38^-$ cells were determined by flow cytometry. The mean frequencies of $CD34^+CD38^-$ cells are shown as a percentage below the treatment for each time point. (C) Effect of Jagged-1 on the total number of clonogenic progenitors. At the times indicated, an aliquot of cells was plated in methylcellulose culture. Total number of CFU per well was determined from the cell input number and number of colonies observed. Values shown are the mean ± SEM ($n = 5$). *Significant difference, $P < 0.05$.

SCID-repopulating cells (SRCs), as demonstrated by initial limiting dilution analysis of de novo (day 0) isolated $CD34^+CD38^-Lin^-$ cells (data not shown). A summary of the frequency of chimeric NOD/SCID mice transplanted with control treated or Jagged-1–treated wells is shown in Fig. 4 A, i and ii. Wells initially seeded with 1,000–2,500 $CD34^+CD38^-Lin^-$ cells and treated with hJagged-1 consistently gave rise to a greater frequency of chimeric NOD/SCID mice as compared with mice transplanted with control treated cells ($n = 89$) (Fig. 4 A, i). To allow for a greater probability of evaluating single SRCs, as few as 1,000–500 $CD34^+CD38^-Lin^-$ cells were initially seeded at day 0 and cultured in the presence or absence of hJagged-1. In these experiments, nearly all human repopulating cells detected were derived only from cultures containing hJagged-1 ($n = 36$; Fig. 4 A, ii). In addition, the mean level of human engraftment (indicated with horizontal bars) was found to be higher in mice transplanted with Jagged-1–treated stem cells (Fig. 4 A, i and ii), suggesting that Jagged-1 treatment before transplantation is capable of enhancing

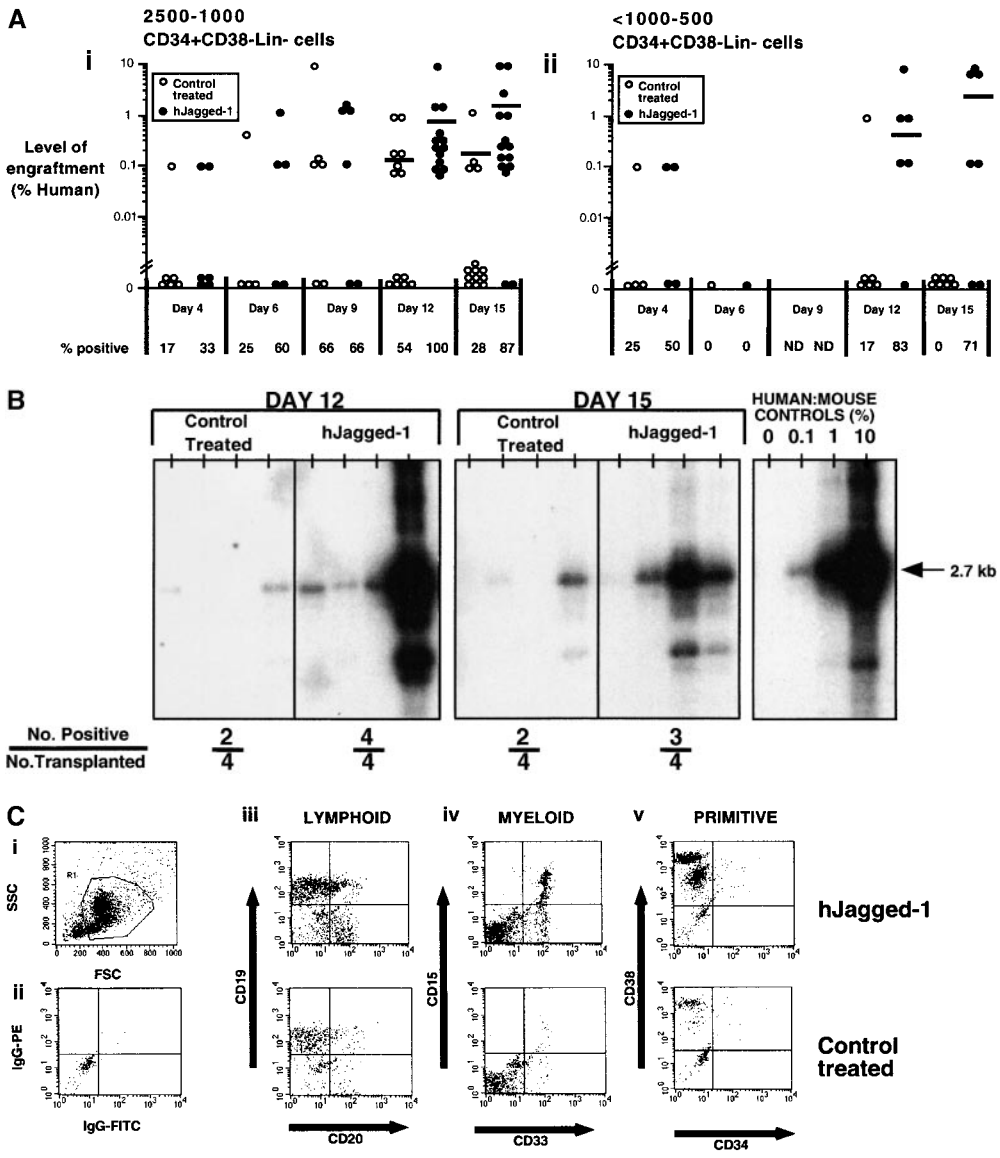

Figure 4. Analysis of engraftment in NOD/SCID mice with $CD34^+CD38^-Lin^-$ cells cultured in the absence or presence hJagged-1. (A) Summary of levels of human cell engraftment in the BM of NOD/SCID mice transplanted with $CD34^+CD38^-Lin^-$ cells cultured in the absence (○) or presence (●) of Jagged-1 (10 μg/ml). Cells were cultured for the different periods indicated and transplanted by tail vein injection into NOD/SCID recipients. (i) Mice injected with 1,000–2,500 $CD34^+CD38^-Lin^-$ cells seeded on Day 0. (ii) Mice injected with 500–1,000 cells seeded on day 0. At days 12 and 15, horizontal bars indicate average level of human chimerism achieved in engrafted mice. (B) Representative Southern blot analysis of individual NOD/SCID mice transplanted with four equally divided aliquots of 2,500 $CD34^+CD38^-$ cells seeded at day 0 and cultured under serum-free conditions for either 12 or 15 d in the presence or absence of hJagged-1. DNA was extracted from recipient murine BM 8 wk after the transplantation, separated on agarose gels, and hybridized with the human chromosome 17–specific α satellite probe. The level of engraftment was determined from the human/mouse DNA controls shown. (C) Multilineage differentiation of human repopulating cells in NOD/SCID mice after ex vivo culture with or without Jagged-1. Cells obtained from the BM of engrafted mice were stained with human specific mAbs and analyzed by flow cytometry. (i) Forward and side scatter properties were used to gate live cells in R1 for analysis. (ii) Isotype control for nonspecific IgG_1 staining for PE and FITC fluorescence. Human cells (positive for panleukocyte marker CD45) were gated and analyzed for expression of the following human markers: (iii) pan-B cell markers for cells of the lymphoid lineage CD19 and CD20, (iv) myeloid markers CD15 and CD33, and (v) CD38 and primitive cell markers CD34. Results are shown for representative mice transplanted with control treated and Jagged-1–treated cultures.

clonal proliferative capacity of human stem cells in vivo. Based on these results, we suggest that hJagged-1, unlike conventional hematopoietic factors, is capable of augmenting the functional capacity of human reconstituting cells.

To further address whether hJagged-1 was capable of expanding human SRCs, purified cells were cultured for 12 and 15 d in the presence or absence of Jagged-1, and harvested cells were divided into four equal aliquots for transplantation into four individual NOD/SCID mice. Using both flow cytometry and a human-specific probe, a representative Southern blot shown in Fig. 4 B illustrates that treatment with Jagged-1 resulted in a greater frequency of human chimeric mice compared with animals transplanted with control treated cultures (Fig. 4 B). To evaluate the in vivo pluripotent capacity of human repopulating cells treated with hJagged-1, mice transplanted with hJagged-1–treated SRCs were analyzed by flow cytometric analysis. Similar to control treated repopulating cells, mice transplanted with hJagged-1–treated stem cells contained human cells of multiple lineages, consisting of both lymphoid and myeloid cells, and primitive $CD34^+$ cells (Fig. 4 C). This analysis reveals that hJagged-1 is capable of acting on human stem cells with pluripotent reconstituting ability, without altering the developmental program or lineage determination of the stem cell in vivo. As greater number of mice were engrafted with hJagged-1–treated cells compared with cells cultured in the presence of control cytokines, we suggest that hJagged-1 is capable of enhancing

the survival and expansion of human reconstituting stem cells. Therefore, unlike previously identified cytokines, our data illustrates that although hJagged-1 has modest effects in modulating mature progenitors detected in vitro (Fig. 3), this ligand represents a novel hematopoietic growth factor capable of expanding human blood cells with in vivo pluripotent reconstituting ability.

Discussion

Due to limitations in harvesting hematopoietic stem cells used for transplantation and the requirement for stem cell self-renewal for retroviral gene transfer in vitro, ex vivo expansion of human hematopoietic cells is a major focus of clinical procedures (5). However, conditions capable of expanding reconstituting cells that do not induce differentiation and loss of stem cell function have yet to be demonstrated using single or combinatory cytokines (4, 5). The cloning, expression, and purification of soluble hJagged-1 has enabled us to treat primary human blood stem cells and define the effect of hJagged-1 for the first time. Although Jagged-1 had little effect on mature progenitor proliferation, the use of a human–mouse xenotransplantation model for human stem cell detection provides the first evidence that addition of a single factor, hJagged-1, can maintain and expand primitive hematopoietic cells capable of multilineage reconstitution in vivo without loss of progenitors. These results indicate that hJagged-1 is unique to any known hematopoietic growth factor tested to date. In addition, as hematopoietic cells themselves, along with stromal microenvironments, have been shown here to express Jagged-1, our results propose a unique paradigm in which stromal and both mature and primitive hematopoietic cells communicate via multidirectional Jagged-1→Notch signaling. The biological effect of hJagged-1 on primitive cells capable of repopulating immunodeficient mice demonstrates that soluble hJagged-1 is a novel hematopoietic stem cell growth factor in the human.

Potential roles of Notch signaling have been indicated in humans through identification of somatic mutations that cause human disease (28). However, segregating Jagged-1 mutations related to highly variable patient haploinsufficient phenotypes, and absence of Jagged-1 expression in effected tissues suggests the role of Jagged-1 is questionable. As it is unknown if mutations in the Notch receptor represent gain or loss of Notch signaling function or if Jagged-1 mutations are a direct cause of disease, genetic analysis has been unable to fully define a role and/or necessity of Notch–ligand interactions in humans (3). Therefore, as our study reveals a novel role of Jagged-1 in human physiology, we suggest that this system provides a manner in which to characterize the biological role of the Notch signaling in humans and suggests that additional functions of Jagged-1 may remain to be demonstrated. For example, transplantation of neural or muscle stem cells has been postulated to have clinical utility in replacing missing cells or the activation of endogenous cells to regenerate new tissue (29, 30) and may eventually revolutionize therapies for neurodegenerative diseases or muscular dystrophy. However, before the full potential of these rare stem cells can be realized, factors that control their proliferation must be identified to expand these cells before implantation. As tissue-specific stem cells may share common embryonic programs in response to environmental stimuli, similar outcomes using hJagged-1 may apply to these tissue-specific stem cells. Based on our current findings, it will be important to examine whether hJagged-1 is capable of acting as a neurogenic or myogenic stem cell regulator.

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