The Cellular Amount of the Common γ-Chain Influences Spontaneous or Induced Cell Proliferation[1]

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Mutations of the *IL2RG* encoding the common γ-chain (γ_c) lead to the X-linked SCID disease. Gene correction through ex vivo retroviral transduction restored the immunological impairment in the most of treated patients, although lymphoproliferative events occurred in five of them. Even though in two cases it was clearly documented an insertional mutagenesis in *LMO2*, it is conceivable that γ_c could have a role per se in malignant lymphoproliferation. The γ_c is a shared cytokine receptor subunit, involved also in growth hormone (GH) receptor signaling. Through short interfering RNA or using X-linked SCID B lymphoblastoid cell lines lacking γ_c, we demonstrate that self-sufficient growth was strongly dependent on γ_c expression. Furthermore, a correlation between γ_c amount and the extent of constitutive activation of JAK3 was found. The reduction of γ_c protein expression also reduced GH-induced proliferation and STAT5 nuclear translocation in B lymphoblastoid cell lines. Hence, our data demonstrate that γ_c plays a remarkable role in either spontaneous or GH-induced cell cycle progression depending on the amount of protein expression, suggesting a potential role as enhancing cofactor in lymphoproliferation. ***The Journal of Immunology,*** **2009, 182: 3304–3309.**

Mutations of the *IL2RG* gene encoding the cytokine receptor common γ-chain (γ_c)[3] lead to the X-linked SCID (X-SCID) disease (1, 2). The severity of this disease makes it a medical emergency, which without any treatment leads to death in the first months of life. Bone marrow transplantation represents in this context the conventional therapeutic strategy for this form of immunodeficiency. This therapeutic approach confers to children affected by SCID at least a 70% chance of cure in the presence of a fully HLA-matched donor. Unfortunately, a fully compatible donor is not always available, thus limiting the successful use of this therapy. Moreover, the use of a not fully HLA-matched donor increases the immunologic complications such as graft-vs-host disease associated with a potential long-term decline in immune cell functions. These difficulties encouraged gene therapy trials (3). This strategy using ex vivo retroviral vectors has been proven as a corrective therapeutic approach for X-SCID in humans (4–9). Immunological reconstitution has been documented in 17 of 20 patients enrolled in two distinct clinical studies (3, 7). Unfortunately, five of these patients developed a lymphoproliferative disorder (10–12), not observed in gene therapy trials for SCID due to adenosine deaminase deficiency (13). This event was attributed to up-regulated expression of the *LMO2* oncogene, as a consequence of insertional mutagenesis (14). However, this event was clearly documented only in two cases. Even though the other patients may have the vector integration near *LMO2* or other oncogenes (14), it is also conceivable that the transgene could have a role per se in cell cycle progression. In keeping with this hypothesis, overexpression of γ_c transduced through a lentiviral vector into stem cells in a murine model of X-SCID led to T cell lymphomas and thymic hyperplasia in a third of the cases. Intriguingly, no common integration site was found between the mice, which developed T cell lymphomas (15). In these mice, differently from humans treated with gene therapy for X-SCID, the expression levels of the protein was elevated thus implying that the amount of the protein may be crucial for the γ_c control of cell cycle (16). These results suggest that insertional mutagenesis may not be the only cause of leukemogenesis and that the expression level of *IL2RG* could influence the cell cycle progression directly or its effect being mediated by cytokines triggers.

The γ_c is a transducing element shared among several IL receptors, whose activity was documented to enhance leukemogenesis (17), and is part of the intermediate- and high-affinity receptor of IL-2, that is essential for ligand internalization (18). In turn, this subunit activates several key signaling molecules such as JAK3, in which constitutive activation is frequently associated to autonomous cell growth and malignant transformation of lymphoid cells (19, 20). Recently, we demonstrated that γ_c subunit is also involved in growth hormone (GH) receptor (GHR) signaling in B lymphoblastoid cell lines (BCLs) (21). GH in BCLs obtained from X-SCID patients was unable to induce cell proliferation and STAT5 activation (22). *IL2RG* gene transduction of X-SCID BCLs promptly restored these functional and biochemical events, eventually resulting in STAT5 nuclear translocation (21).

In this study, we show through γ_c silencing experiments that the molecule is actively involved in a concentration dependent manner in self-sufficient growth and GH-induced cell cycle progression of BCLs, its activation being mediated by STAT5 phosphorylation and nuclear translocation.

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[3] Abbreviations used in this paper: γ_c, common γ-chain; GH, growth hormone; GHR, GH receptor; BCL, B lymphoblastoid cell line; siRNA, small interfering RNA; X-SCID, X-linked SCID.

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Materials and Methods

Reagents

Recombinant human GH was obtained from Serono. The ECL kit was purchased from Amersham Biosciences. The Abs anti-γ_c, anti-JAK3, anti-β-actin, anti-histone 3 (H3), anti-phosphotyrosine, anti-STAT5 were purchased from Santa Cruz Biotechnology. The neutralizing anti-IL-2 and anti-IL-4R mAbs were purchased from R&D Systems. Acrylamide and bisacrylamide were obtained from Invitrogen. Prestained molecular mass standards were obtained from Bio-Rad. The small interfering RNA (siRNA) duplexes specific for γ_c and the control nontargeting siRNA were obtained from Invitrogen. The control nontargeting pool contains nontargeting siRNAs with guanine cytosine content comparable to that of the functional siRNA but lacking specificity for known gene targets. Except where noted, other reagents were from Sigma-Aldrich.

Cells and cell cultures

Mononuclear cells (PBMC) were obtained from four X-SCID patients and six normal donors of heparinized peripheral blood by Ficoll-Hypaque (Biochrom) density gradient centrifugation (21). BCLs were generated by EBV immortalization of patients and control PBMC using standard procedures. Cells were maintained in RPMI 1640 (Biochrom) supplemented with 10% FBS (Invitrogen), 2 mM/L L-glutamine (Invitrogen), and 50 μg/ml gentamicin (Invitrogen), and cultured at 37°C, 5% CO_2. In self-sufficient growth experiments, BCLs were cultured in DMEM/F12 without FBS and supplemented with 2 mM/L L-glutamine.

In neutralization experiments, BCLs were cultured in 96-well plates, preincubated with the neutralizing mAbs 202 or 230 at the indicated concentrations.

siRNA transfection

Preparation of the cells before Lipofectamine 2000 transfection was performed according to the manufacturer's recommendations. Briefly, for each transfection 1×10^6 BCLs in 1 ml were treated with 20 μl of 50 μM siRNAs specific for the γ_c or equal amount of the control nontargeting siRNA. The siRNAs were solubilized and formed complexes separately with the lipid-based transfectant, Lipofectamine 2000. The siRNA-lipofectamine complexes were transfected into the cultured cells in a 24-well plate and incubated for the time indicated in the text. Throughout the experiments, cell vitality was monitored continuously by trypan blue exclusion assay. Furthermore, 96 h after the transfection, the cells were washed, placed in fresh culture medium and used for further analysis, as described.

CFSE labeling

Cell proliferation was measured by the cell surface stain CFSE. BCLs (1×10^6) were labeled with 1.7 μM CFSE in PBS just before culturing for the indicated times using a serum-free medium. After 2 min at room temperature, BCLs were washed in FBS and PBS and cell division accompanied by CFSE dilution was analyzed on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences).

[^{3}H]thymidine incorporation assay

Cell number was assessed by counting cells after trypan blue dye exclusion staining. BCLs were cultured for different time ranging between 6 h and 4 days at a density of 1×10^5 viable cells/200 μl well in triplicate wells (96-well microtiter plates, Falcon; BD Biosciences). Cultures were pulsed with 0.5 μCi [^{3}H]thymidine for 8 h (or 6 h in the short-term cultures) before harvesting and the incorporated radioactivity measured by scintillation counting. Where indicated (see Fig. 5), recombinant GH was added to the culture at 50 ng/ml. The results are expressed as mean cpm for triplicate cultures.

Immunoprecipitations and Western blotting

Following transfection and appropriate recombinant GH stimulation, BCLs were lysed in 100 μl of lysis solution (20 mM Tris (pH 8), 137 mM NaCl, 1% Nonidet P-40, 10 mM EDTA, 1 mM PMSF, 1 mM sodium orthovanadate, 5 μg/ml leupeptin, and 5 μg/ml aprotinin) on ice for 45 min. Protein concentration was determined by Bio-Rad protein assay. The cell lysates were stored at −80°C for Western blot analysis. Nuclear extracts were prepared by the method previously described (21). Proteins were electrophoretically separated on 10% Tris glycine SDS-PAGE gels. Proteins were transferred onto nitrocellulose transfer membranes (Schleicher & Schuell). Membranes were incubated with the specific primary Abs. Immune complexes were detected using the appropriate anti-rabbit or anti-mouse peroxidase-linked Abs. ECL detection system was used for visualization.

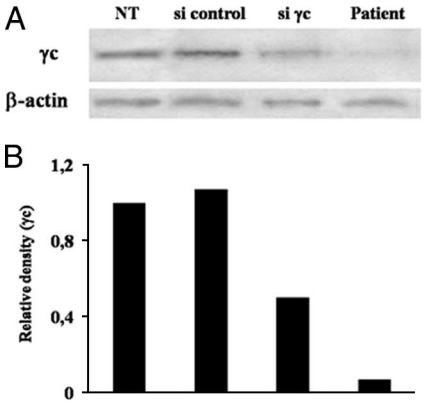

FIGURE 1. The γ_c silencing by siRNA induced a reduction in protein amount. *A*, After 96 h of culture, control BCLs transfected with nontargeting siRNA (si control), γ_c siRNA (si γ_c), or nontransfected (NT), and X-SCID BCLs (patient) were lysed and γ_c total amount was measured by Western blotting. Membranes were incubated as indicated with Abs anti-γ_c and anti-β-actin, used as loading control. *B*, Densitometric analysis of the above Western blot. ImageJ program was used to generate the data.

Equal loading was confirmed after stripping and reprobing with anti-β-actin or anti-histone 3 Abs.

For immunoprecipitation, lysates were normalized for either protein content or cell number and precleared with protein G-agarose beads (Amersham Biosciences). The supernatant was incubated with 2 μg/ml anti-JAK3 or polyclonal serum, followed by protein G-agarose beads. The immunoprecipitates were separated on density gradient gels, followed by Western blotting. Proteins were detected using Ab for phosphotyrosine.

Densitometric analysis was performed on a Windows personal computer, using the public domain Java image processing program ImageJ (developed at the National Institutes of Health and at (http://rsb.info.nih.gov/ij/index.html). Each signal has been evaluated in comparison with the control lane 1 and equalized for the loading control, applying the following formula: (sample lane/control lane)/loading control ratio.

Confocal microscopy

After appropriate stimulation, as indicated, cells were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.0) for 30 min at room temperature and centrifuged in a Shandon Cytospin III (Histotronix) onto a glass slide and permeabilized by incubation in a 0.2% Triton X-100 solution for 20 min (21). BCLs were incubated for 1 h at room temperature with rabbit anti-STAT5 Ab in PBS containing 1% BSA. After four washings for 5 min in PBS, the cells were incubated for 1 h at room temperature with FITC-conjugated donkey anti-rabbit IgG (Pierce) in PBS. After washing in PBS, the glass slides were mounted under a coverslip in a 5% glycerol PBS solution. The slides were analyzed by laser scanning confocal microscopy using a Zeiss LSM 510 (version 2.8 SP1 Confocal System). At least 100 cells per condition were analyzed in each experiment to determine the rate of STAT5 nuclear translocation.

Results

Common γ_c silencing inhibits self-sufficient growth and down-regulates constitutively activated JAK3 in B cell lines

To define an intrinsic mitogenic property of γ_c dependent on the amount of the protein, we used in vitro cellular models containing different amounts of γ_c. In particular, BCLs from normal subjects, cells transduced with lipid vector containing nontargeting siRNA, BCLs transduced with siRNA to knockdown γ_c expression and BCLs from X-SCID patients were used. The transfection efficiency was tested using fluorescent oligonucleotides under fluorescent microscope. Levels of γ_c were evaluated by Western blotting of whole cell lysates. The γ_c expression was reduced to 50% of the control in γ_c-silenced BCLs and completely undetectable in X-SCID BCLs (Fig. 1*A*). Densitometric analysis is shown in the histogram in Fig. 1*B*.

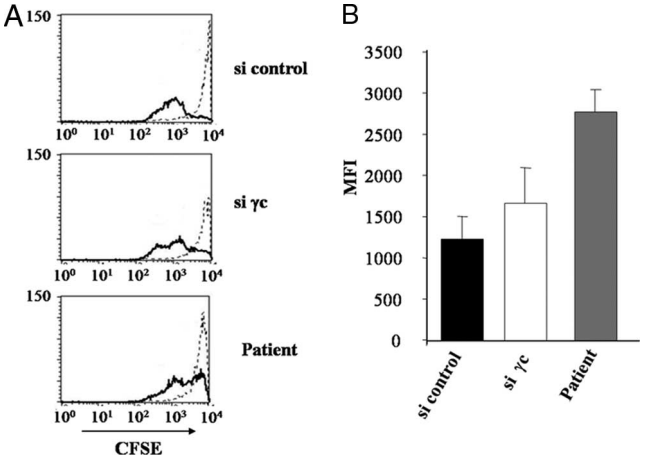

FIGURE 2. The γ_c was involved in self-sufficient growth of BCLs. *A*, After 12 h of starvation, BCLs transfected with control nontargeting siRNA (si control) or γ_c siRNA (si γc) and X-SCID BCLs (patient) were stained with 1.7 μM CFSE and cultured in the absence of serum. After 6 h, cells were harvested and cell proliferation was assessed using cytofluorimetry for CFSE intensity. CFSE dilution profiles are shown. Histograms show on gated cells the number of events (*y*-axis) and the fluorescence intensity (*x*-axis) 6 h following the start of the culture. Dashed lines represent the start of the culture. *B*, Mean fluorescence intensity (MFI) of gated CFSE-positive cells maintained in the same conditions as described in *A*.

Because self sufficiency in growth has been suggested as one of the six acquired capabilities of cancer phenotype (23), we examined the abilities of these previously mentioned BCLs to grow in serum-deficient conditions, upon trypan blue exclusion assay. We labeled BCLs, after 12 h of starvation, with CFSE, a dye that allows proliferative history to be visualized, and assessed the

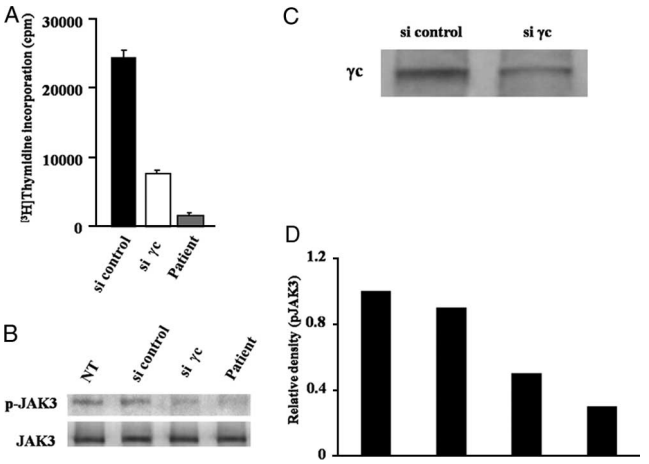

FIGURE 3. The γ_c protein depletion had effect on spontaneous cell proliferation and on activated JAK3 levels. *A*, BCLs transfected with control nontargeting siRNA (si control) or γ_c siRNA (si γc) and X-SCID BCLs (patient) were tested for their ability to proliferate in serum-free medium. Cultures were maintained in serum-free medium for 4 days and pulsed with [^{3}H]thymidine for the final 8 h. Radioactive incorporation was counted. Error bar indicates 1 SD. *B*, Unstimulated BCLs, after 12 h of starvation, were immunoprecipitated with anti-JAK3 Ab and tested in Western blot with anti-phosphotyrosine mAb. Equivalent loading was controlled by reprobing the membrane with JAK3 Ab. Nontransfected (NT) BCLs were also tested. *C*, Control BCLs, transfected with nontargeting siRNA (si control) and γ_c siRNA (si γc), were lysed and γ_c total amount was measured by Western blotting. *D*, Densitometric analysis of Western blot shown in *B*.

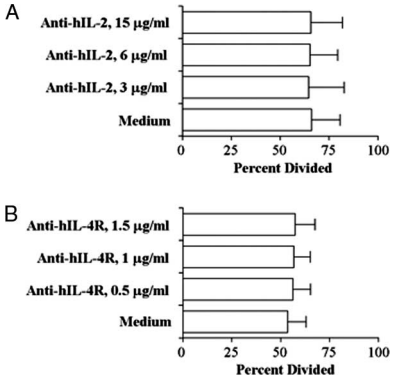

FIGURE 4. The γ_c-activating cytokines did not affect self-sufficient growth of BCLs. *A*, Control BCLs were treated with the indicated concentration of Anti-human IL-2, were stained with 1.7 μM CFSE and cultured in the absence of serum. After 6 h, cells were harvested and cell proliferation was assessed using cytofluorimetry for CFSE intensity. *B*, Control BCLs were treated with the indicated concentration of anti-human IL-4R and analyzed as described in *A*. The percentage of cells that divided is shown. Error bar indicates 1 SD.

CFSE dilution profile at different time points ranging between 6 h and 7 days to establish the rate of spontaneous cell proliferation (data not shown). Informative data on differences between previously described BCLs were appreciable as soon as 6 h from the start of the culture, presumably because of the high proliferation rate of BCLs as compared with normal mononuclear cells. At this time, only 14% of control cells retained the dye, indicating a high proliferation rate, compared with 26% of γ_c-silenced cells and to 50% of X-SCID BCLs (Fig. 2*A*). In addition, the final mean fluorescence intensity, reflecting CFSE-derived fluorescence per cell, were 1378 units in control, 1825 in γ_c-silenced, and 2866 in X-SCID, thus confirming that only in control cells a substantial dye dilution occurred (Fig. 2*B*).

We then cultured viable cells for 4 days in a serum free medium and 0.5 μCi of [^{3}H]thymidine were added 8 h before harvesting. γ_c-silencing reduced cell proliferation of unstimulated BCLs by 69% as compared with control cells. In X-SCID BCLs the extent of the reduction was higher corresponding to 97% of control BCLs (Fig. 3*A*). These data were, therefore, in keeping with the results of CFSE experiments. Moreover, to prove that the effect observed in the CFSE experiments in the 6 h cultures were really indicative of cell proliferation, several time-course experiments with both techniques were performed at the beginning of the study. These data indicate that the proliferation rate of these cells is comparable using the two methods in the first 12 h (see supplemental materials S1 and S2),[4] indicating that the CFSE dilution reflects a real cell division. Furthermore, the addition of mitomycin prevents staining dilution, providing further evidence that CFSE signals reflect a real cell division. In particular, after 6 h of culture 12, 31, and 59% of the control, silenced or SCID patient cells, respectively, retained the dye as compared with the 100% of stained cells at the beginning of the culture (see supplemental material S3).[4]

Because JAK3 is essential for autonomous proliferation being physically linked to γ_c, we further investigated the role of γ_c in self-sufficient growth, evaluating JAK3 activation. Of note, JAK3 proteins are constitutively phosphorylated in EBV-immortalized B cells and other malignant cells (24). Thus, we evaluated the effect of different amount of γ_c on the levels of constitutively phosphorylated JAK3 protein (phospho-JAK3). After 12 h of serum-free

[4] The online version of this article contains supplemental material.

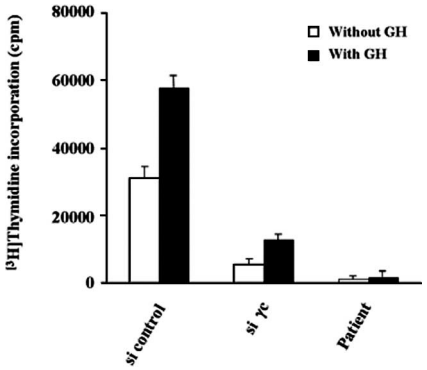

FIGURE 5. Silencing of γ_c inhibited GH-induced proliferation. Cell proliferation of BCLs transfected with control nontargeting siRNA (si control) or γ_c siRNA (si γc) or nontransfected (NT) stimulated with recombinant GH (50 ng/ml) was evaluated through [^{3}H]thymidine incorporation assay. Error bar indicates 1 SD.

culture, whole cell lysates were immunoprecipitated with anti-JAK3 Ab and the obtained membranes were immunoblotted with anti-phosphotyrosine mAb. A higher constitutive activation of JAK3 was found in control BCLs, whereas a decrease in phospho-JAK3 levels was observed in γ_c-silenced and in X-SCID BCLs, despite a comparable amount of the whole protein (Fig. 3*B*). The amount of pJAK3 paralleled the amount of γ_c, shown in Fig. 3*C*. The densitometric analysis of phospho-JAK3 equalized for total JAK3 is shown in a histogram in Fig. 3*D*.

Evidence is available that γ_c-dependent cytokines, as IL-2 and IL-4, may be secreted in EBV-infected B cells (25–27). Thus, to define whether the mitogenic effect of γ_c was independent or dependent from receptor engagement of these endogenous γ_c-activating cytokines, we used neutralizing mAbs anti-IL-2 or anti-IL-4R in the CFSE-based proliferative assay. As shown in the Fig. 4, the neutralizing mAbs did not reduce at any concentration spontaneous cell proliferation.

The γ_c silencing inhibits GH-induced cell proliferation and subsequent STAT5 activation

It has been reported that GH enhances BCLs proliferation in vitro (28) and that γ_c is functionally linked to GHR (21). Moreover, it has been described the association between lymphoproliferative events and supraphysiological doses of GH, both in mice and humans (29).

Because growth factors may participate in autocrine or paracrine loops that affect tumor cells growth or survival and autocrine production of GH is able to induce cellular transformation (30), we evaluated the response to GH stimulation of X-SCID BCLs, control cells and γ_c-silenced BCLs to assess whether γ_c amount could influence GH response. Recombinant GH at a concentration of 50 ng/ml enhanced proliferation of control BCLs. In γ_c-silenced or X-SCID BCLs, recombinant GH induced proliferation at a much lower extent, corresponding to 28% and 5% of the control, respectively (Fig. 5).

Because the activation of JAKs and STATs represents a prominent biochemical event during GH-dependent proliferation of lymphoid cell lines (31–35) and STAT5 is considered a transforming agent in lymphoma and other cell types (36), we then evaluated whether γ_c-silencing had effect on GH-induced STAT5 subcellular localization. Nuclear and cytoplasmic extracts from BCLs, unstimulated or treated with 500 ng/ml recombinant GH, were evaluated by immunoblot for the overall amount of STAT5. Recombinant GH induced a rapid decrease of the cytoplasmic amount of STAT5 in control BCLs and in BCLs treated with control nontargeting siRNA, differently from γ_c-silenced BCLs, in which no effect on the protein amount was observed (Fig. 6*A*). This finding was inversely correlated with the amount of the nuclear form of the molecule. In fact, in control BCLs and in BCLs treated with control siRNA, an increase of nuclear STAT5 amount was observed after recombinant GH stimulation, differently from what observed in γ_c-silenced BCLs, in which no change was observed (Fig. 6*C*). These data, representative of different experiments, reflect a real subcellular redistribution of the molecule in that no difference in the cytoplasmic β-actin and nuclear histone H3 expression was observed. The densitometric analysis normalized for the housekeeping molecules is shown in Fig. 6, *B* and *D*.

Furthermore, we looked at STAT5 subcellular localization using confocal microscopy. In control unstimulated BCLs, only 10% of cells showed a nuclear localization of STAT5, being the protein mainly concentrated in the cytoplasm. GHR perturbation through recombinant GH stimulation at a concentration of 500 ng/ml

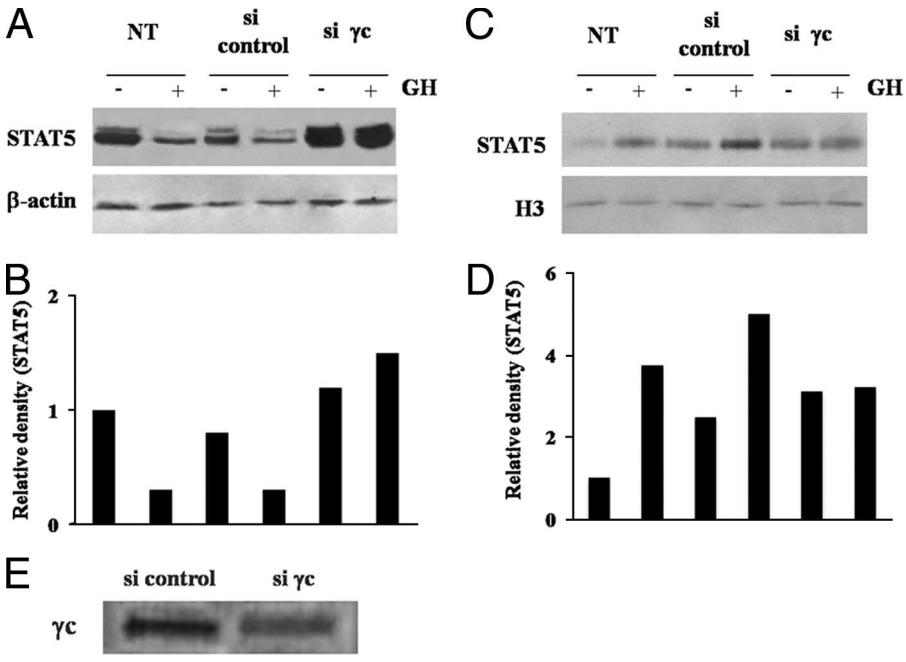

FIGURE 6. Silencing of γ_c influenced STAT5 nuclear translocation in B cell lines. Cells from BCLs transfected with control nontargeting siRNA (si control) or γ_c siRNA (si γc) and X-SCID BCLs (patient) were analyzed for subcellular localization of STAT5. Cells were stimulated with 500 ng/ml recombinant GH for 30 min. *A*, Cytoplasmic amount of STAT5. Equivalent loading was controlled by reprobing the membrane with β-actin. *C*, Nuclear fraction of STAT5. Equivalent loading was controlled by reprobing the membrane with histone H3. *B* and *D*, Densitometric analysis of the Western blots from BCLs in *A* and *C*. *E*, Control BCLs, transfected with nontargeting siRNA (si control) and γ_c siRNA (si γc), were lysed and γ_c total amount was measured by Western blotting.

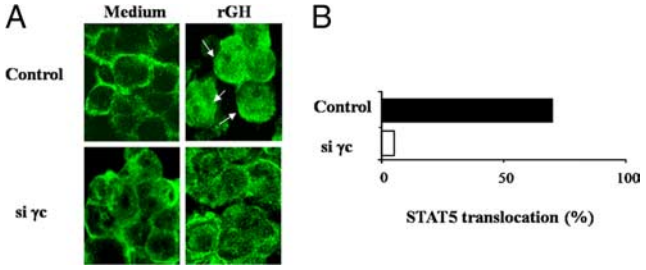

FIGURE 7. The γ_c silencing impairs recombinant GH-induced STAT5 subcellular redistribution. *A*, Evaluation of STAT5 subcellular localization through confocal microscopy. Control or γ_c-silenced BCLs (si γc) were cultured in the absence or presence of 500 ng/ml recombinant GH for 30 min. Arrows indicate exemplificative cells with nuclear STAT5 staining. Nucleoli are not stained. *B*, The percentage of STAT5 nuclear translocation is shown. These data represent an analysis of independent observations.

induced STAT5 nuclear localization in the 70% of cells. Differently, recombinant GH stimulation of γ_c-silenced BCLs had negligible effects on nuclear STAT5 migration, resulting in a 5% increase of positively stained cells as compared with unstimulated cells (Fig. 7).

Discussion

Our results indicate that silencing of γ_c induces a substantial decrease of protein amount in BCLs, that allowed us to demonstrate a direct involvement of γ_c in self-sufficient growth of BCLs in a concentration dependent manner. Moreover, we documented that the amount of γ_c also influences the response of BCLs to GH-induced proliferation and STAT5 subcellular redistribution that follows GHR perturbation. These data add new evidence for a possible intrinsic mitogenic role of γ_c related to its cellular amount. This biologic effect could be either direct, thus related to the molecule per se, or indirect and mediated by the participation to cytokine-receptors signaling.

The intrinsic property of γ_c in cell cycle progression has been long debated. In fact, although gene therapy trials have been proved as a beneficial alternative approach to cure X-SCID patients carrying mutations of γ_c, a malignant lymphoproliferation occurred in 5 of 20 patients enrolled into the trials, alarming the scientific community (3). To explain these adverse events, studies were conducted to define whether the retroviral insertional mutagenesis could have played a role. In two cases, an aberrant transcription and expression of *LMO2* was clearly documented (14). However, for the remaining patients there isn't any evident demonstration of *LMO2* alteration due to random insertions that could be causative in transformation. An in vivo expansion of cell clones has also been documented in other gene therapy trials. Two patients treated with gene therapy for X-linked chronic granulomatous disease developed myeloid proliferation. Of note, in these cases cell clones didn't exhibit any self-renewal capacity. This observation would imply that there is no evidence of continued abnormal growth of clones containing insertionally activated growth-promoting genes (37). Of note, development of leukemia, similar to other cancers, requires multiple genetic changes caused by a diverse group of genes that inhibit apoptosis or provide growth advantage to the leukemic cells (38). In this study, we demonstrate that γ_c exerts a role in cell cycle progression in a strictly concentration dependent manner. We, also, found that the amount of constitutively activated JAK3 parallels the extent of γ_c expression. This finding is intriguing, in that constitutively active or hyperactive JAK proteins have crucial roles in hematopoietic malignancies, by promoting oncogenic transformation and uncontrolled blood cell production (17). In particular, JAK overexpression can be considered as one of the main biologic events leading to the constitutive activation of the JAK-STAT pathway, that contributes to oncogenesis (20). In lymphoid cells, the involvement of the JAK/STAT pathway in several cellular processes, such as proliferation and protection from apoptosis, has also been well documented (39, 40). Moreover, the role of JAK3 in cell destiny is emphasized by the finding that JAK3 mutations cause a SCID phenotype, thus implying its role in lymphoid development (41). JAK3 has also the capacity to activate DNA synthesis and protooncogenes, such as c-*myc* and c-*fos* (42).

In this study, we also observed that the participation of γ_c in GHR signaling apparatus and, in particular, in GH-induced STAT5 activation and nuclear translocation was also dependent on the extent of its molecular expression. Thus, the concentration-dependent mitogenic effect of γ_c could be favored by the participation of γc in GHR signaling. Of note, it should be mentioned that experimental studies document a role for GH in the initiation or promotion of tumorigenesis, raising the possibility that patients treated with GH might be at increased risk of cancer (29). Moreover, a putative role of GH as a cofactor in tumor growth is plausible because several carcinomas express GHR (43). In animal models, GH increase the incidence of leukemia and solid tumors, and in humans, at supraphysiological doses, it can promote lymphoproliferative events (44).

Our data would imply that the expression levels of γ_c in hematopoietic cells are crucial for the maintenance of cell growth control. Whether our data may have direct implications in the understanding of the pathogenesis of the lymphoproliferative events occurring during gene therapy trials for X-SCID remains to be clarified. Even though, under ordinary conditions, γ_c is expressed at a normal extent in cells, transduced with retroviral vectors containing wild-type γ_c, our data indicate that altering the expression levels of the protein could be important in modifying cell cycle control mechanisms. Our findings are in keeping with a recent study, which demonstrates that in X-SCID murine model, T cell lymphomas and thymic hyperplasia occur in a third of the cases treated with lentiviral vectors containing wild-type γ_c (15). This event was independent from common integration sites and, thus, not attributable to insertional mutagenesis, but rather to an intrinsic oncogenic property of the transgene and, presumably, to the overexpression of the molecule (15). Furthermore, by searching in Mouse Retroviral Tagged Cancer Gene Database, integration in *Il2rg* has been found in two cases of retrovirally induced leukemias (45).

In conclusion, our data demonstrate a direct relationship between the amount of γ_c expression and its role in cell cycle progression. Therefore, because results of gene therapy trials for X-SCID have been very promising, to achieve safer results, the modulation of the transgene expression could help reduce the risk of undesirable events.

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Disclosures

The authors have no financial conflict of interest.

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