Gain-of-function mutations in *interleukin-7 receptor-α* (*IL7R*) in childhood acute lymphoblastic leukemias

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Interleukin-7 receptor α (_IL7R_) is required for normal lymphoid development. Loss-of-function mutations in this gene cause autosomal recessive severe combined immune deficiency. Here, we describe somatic gain-of-function mutations in _IL7R_ in pediatric B and T acute lymphoblastic leukemias. The mutations cause either a serine-to-cysteine substitution at amino acid 185 in the extracellular domain (4 patients) or in-frame insertions and deletions in the transmembrane domain (35 patients). In B cell precursor leukemias, the mutations were associated with the aberrant expression of cytokine receptor-like factor 2 (CRLF2), and the mutant IL-7R proteins formed a functional receptor with CRLF2 for thymic stromal lymphopoietin (TSLP). Biochemical and functional assays reveal that these _IL7R_ mutations are activating mutations conferring cytokine-independent growth of progenitor lymphoid cells. A cysteine, included in all but three of the mutated IL-7R alleles, is essential for the constitutive activation of the receptor. This is the first demonstration of gain-of-function mutations of _IL7R_. Our current and recent observations of mutations in _IL7R_ and _CRLF2_, respectively suggest that the addition of cysteine to the juxtamembranous domains is a general mechanism for mutational activation of type I cytokine receptors in leukemia.

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Abbreviations used: ALL, acute lymphoblastic leukemia; BCP-ALL, B cell precursor acute lymphoblastic leukemia; CCR, continuous complete remission; CRLF2, cytokine receptor-like factor 2; DS, Down syndrome; DS-ALL, Down syndrome with BCP-ALL; IGHα, immunoglobulin heavy locus; IL2RG, IL-2 receptor γ; RPS6, ribosomal protein S6; STAT5, signal transducer and activator of transcription 5; TSLP, thymic stromal lymphopoietin.

IL-7R α (*IL7R*) is required for normal lymphoid development (Peschon et al., 1994). Loss-of-function mutations in this receptor cause severe combined immune deficiency (OMIM 608971; Puel et al., 1998). IL-7R heterodimerizes either with IL-2R γ (IL2RG) to form a receptor to IL-7 or with cytokine receptor-like factor 2 (CRLF2) in the receptor for thymic stromal lymphopoietin (TSLP; Noguchi et al., 1993; Liu et al., 2007). TSLP is a cytokine that mediates inflammation and allergy expressed on macrophages and T cells (Liu et al., 2007). Signaling from the TSLP receptor activates signal transducer and activator of transcription (STAT5) by phosphorylation of JAK1 and JAK2 through association with IL-7R and CRLF2, respectively (Rochman et al., 2010).

We and others have recently described the aberrant expression of CRLF2 in B cell precursor acute lymphoblastic leukemia (BCP-ALL) caused by genomic aberrations (Mullighan et al., 2009;

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Russell et al., 2009; Hertzberg et al., 2010; Yoda et al., 2010). These are either translocations into the immunoglobulin heavy locus or interstitial deletions creating chimeric *P2RY8-CRLF2* transcripts. Additional mutations in the pathway, most commonly in *JAK2* but occasionally in *JAK1* or in *CRLF2* itself, occur in approximately half of the patients. Overall, CRLF2 deregulation occurs in 5–10% of childhood ALL and in 60% of children with Down syndrome and BCP-ALL (DS-ALL; Izraeli, 2010) and is associated with a worse prognosis (Cario et al., 2010; Harvey et al., 2010; Yoda et al., 2010; Ensor et al., 2011). Importantly, these leukemias may be sensitive to JAK inhibitors, suggesting the potential for a targeted therapy.

IL7R is constitutively expressed in BCP-ALL, and the formation of a TSLP receptor is plausible in cases with aberrant expression of *CRLF2* (Hertzberg et al., 2010). Receptor association is functional because primary BCP-ALL cells expressing CRLF2 respond to TSLP, but not to IL-7, by phosphorylation of STAT5 and RPS6 (Tasian et al., 2010). We therefore hypothesized that *IL7R* might be mutationally activated in ALL samples expressing CRLF2. In addition to confirmation of this hypothesis, we also report the identification of such mutations in 10% of T-ALLs, thereby demonstrating a general involvement of the IL-7R in ALL.

RESULTS AND DISCUSSION

We screened DNA derived from 133 bone marrow samples of BCP-ALLs, including 62 DS-ALLs, with aberrant expression of CRLF2 and additional 153 BCP-ALL not expressing CRLF2 including 21 DS-ALLs. Nine leukemias with *IL7R* mutations were identified. The rate of mutations in the CRLF2 group (8/133; 6%) was significantly higher than the rest of BCP-ALLs (1/153; 0.6%; $P = 0.01$, Fisher's exact test; Table I).

Two types of mutations in *IL7R* were identified (Fig. 1, A and B, and Table I). Replacement of serine with cysteine at position 185 at the extracellular domain in four patients and complex in-frame insertions and deletions resulted in the addition of 3–7 aa at the transmembrane domain in 5 patients. Whereas the inserted amino acids varied from patient to patient, cysteine was always included (Fig. 1 C).

All mutations were heterozygous and somatic, as they were absent in remission bone marrows. The mutated mRNA was expressed (Fig. 1 D and Fig. S1). *JAK2* mutations were present in three samples, whereas no mutations in JAK1 were identified. Four patients relapsed. Examination of DNA derived from matched diagnostic and relapse samples revealed clonal diversification. The same *IL7R* mutation was present in both diagnosis and relapse in patient M61. *IL7R* mutation was present only at relapse in patient M122, whereas *CRLF2* expression was already noted at diagnosis, suggesting that *IL7R* mutation was a progression event. Conversely, in two patients, M90 and M112 *CRLF2* abnormalities and *IL7R* mutations were present at diagnosis and not in relapse, suggesting that relapse arose from a different subclone. These findings are consistent with the recent studies on the marked clonal diversity of B- and T-ALLs (Rothman et al., 2005; Anderson et al., 2011; Clappier et al., 2011).

We next tested whether the somatic mutations in IL-7R are gain-of-function mutations that cooperate with CRLF2 to form a constitutively active TSLP receptor. We used the IL-3–dependent mouse pro–B cells BaF3 (not expressing endogenous TSLP receptor; Fig. S2) to generate cell lines that express either WT or mutated *IL7R* alone or together with *CRLF2*. We expressed IL-7R S185C and the c.819 Ins12 (CCCCCGTGCCTA) 243 insertion (Ins) PPCL (herein "InsPPCL") representing the two types of mutations. All proteins were expressed at the cell membrane as demonstrated by flow cytometry (Fig. 2 A). 1 wk after IL-3 withdrawal, only cells transduced with CRLF2 and mutated IL-7R survived (Fig. 2, B and C). Closer examination revealed two populations of surviving BaF3 cells transduced with CRLF2 and IL-7R InsPPLC, suggesting that IL-7R InsPPLC provided survival advantage by itself. Indeed, growth assays of BaF3 cells transduced with the IL-7R InsPPLC construct in the absence of CRLF2 (Fig. 2 B) revealed robust cytokine-independent growth. In contrast, BaF3 cells expressing IL-7R S185C required CRLF2 co-expression for survival. Cells expressing only CRLF2, IL-7R S185C, or WT CRLF2 and WT IL-7R did not grow in the absence of cytokines.

Table I. Patients with B cell precursor ALL and somatic mutations in *IL7R*

ID	*IL7R* mutation DNA	IL-7R mutation protein	CRLF2	JAK2	Gender	Age at Dx	WBC/liter	Events
						yr		
DS46	c.819 Ins 12	243 InsPPCL	P2RY8-CRLF2	mut	M	4.9	30.5×10^9	first CCR
DS92	c.642 A>T	S185C	IGHα translocation	WT	M	15.1	112.8×10^9	first CCR
M90	c.828 Ins7 Del T	246 InsKCH	P2RY8-CRLF2	WT	M	1.7	123×10^9	relapse
M112	c.642 A>T	S185C	P2RY8-CRLF2	mut	F	14.3	9.7×10^9	relapse
M117	c.814 Ins13 Del A	241 InsFSCGP	P2RY8-CRLF2	mut	F	10.3	7×10^9	first CCR
M122	c.642 A>T	S185C	P2RY8-CRLF2	WT	M	7.0	7.3×10^9	relapse
M124	c.820 Ins10 Del C	244 InsCHL	High expression	WT	M	4.9	8.9×10^9	first CCR
M223	c.642 A>T	S185C	P2RY8-CRLF2	WT	M	8.0	27×10^9	first CCR
M61	c.820 ins21	244 InsPPVCSVT	CRLF2 not expressed	WT	M	10.7	152×10^9	relapse

DS, Down syndrome; M, male; F, female; mut, mutated at JAK2 R683; CCR, continuous complete remission; Dx, Diagnosis; Del, Deletion; Ins, Insertion; WBC, white blood cells count.

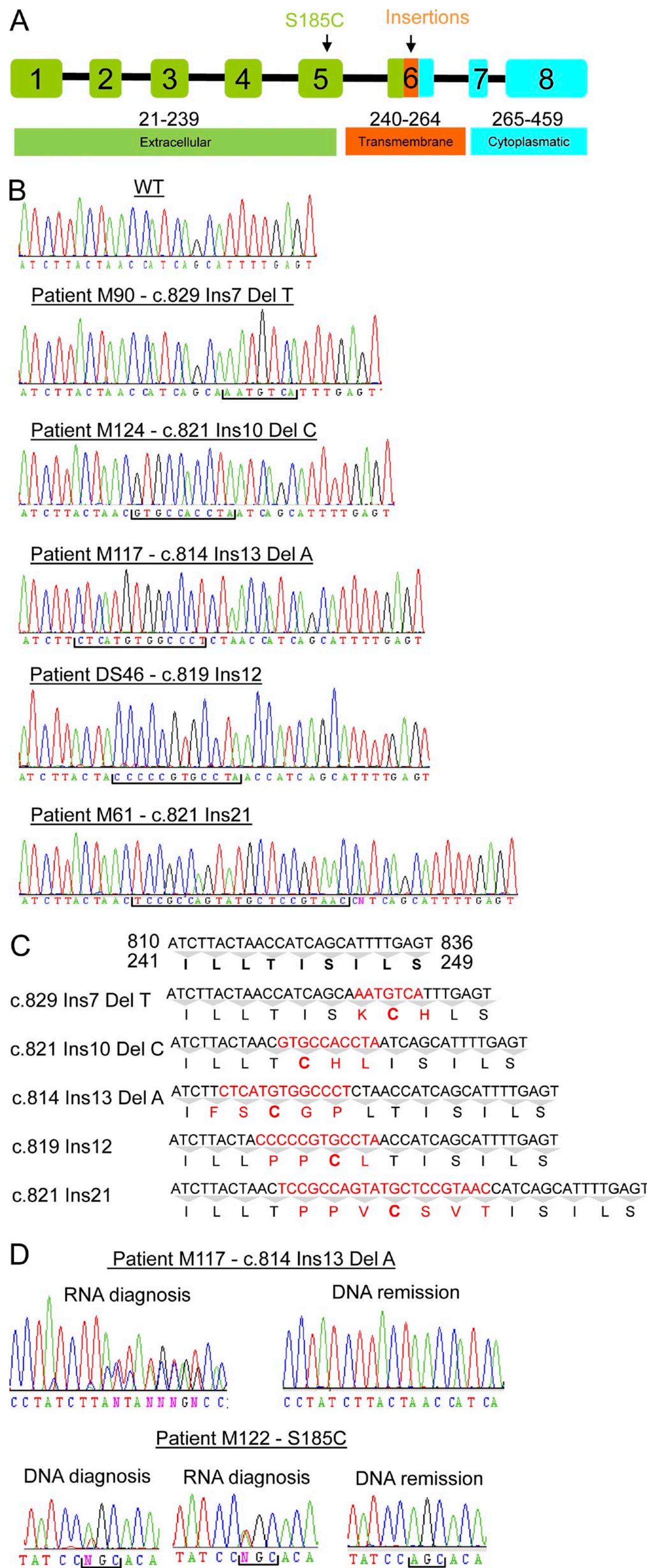

Figure 1. Somatic mutations of *IL7R* (NM_002185.2) in patients with BCP-ALL. (A) *IL7R* mutation localization. Amino acid numbers of the different domains are indicated. (B) Sequences of *IL7R* insertions and deletions mutations at the transmembrane domain. The inserted sequences are within brackets (All Ins-Del mutations were heterozygous, only the mutated allele is shown after allele separation). N, sites with both WT and mutant allele at the same position; Ins, insertion; Del, deletion. (C) Alignment of WT and mutated *IL7R* transmembrane domain sequences. Numbers show the positions of nucleotides and corresponding amino acids. The inserted amino acids are shown in red, with the cysteine residue in bold. (D) Expression of mutations: Examples of two mutated IL-7R sequences with S185C and c.814 Ins 13 Del A. The mutated allele is expressed in the RNA from diagnosis, but not in remission samples.

Biochemical analysis of proteins extracted from BaF3 cell was consistent with the growth assays (Fig. 2 C). Both Stat5 and RPS6 were phosphorylated in the absence of cytokine in BaF3 cells transduced with the IL-7R InsPPCL alone, but not in cells expressing IL-7R S185C or WT IL-7R. Co-expression of CRLF2 with each of these mutated IL-7R proteins, but not with the WT IL-7R caused constitutive phosphorylation of Stat5 and RPS6. Together, the functional and biochemical assays demonstrate that the two types of somatic mutations in *IL7R* are activating mutations causing cytokine independent growth of mouse pro–B cells and constitutive activation of STAT and mTOR pathways. In accordance with the functional studies, the single patient in whom *IL7R* was mutated in the absence of CRLF2 expression (Table I, patient M61) had the insertion type of mutation with the CRLF2-independent activation phenotype.

To see if CRLF2 and mutated IL-7R form a functional TSLP receptor, BaF3 cells expressing CRLF2 and either WT or mutated IL-7R were starved from IL-3 and treated with 100 ng/ml TSLP for 25 min. As noted in Fig. 2 C, cells expressing the mutated receptor exhibited Stat5 and RPS6 phosphorylation in the absence of cytokine. Yet all cells responded to TSLP with a marked increase in Stat5 and RPS6 phosphorylation (Fig. 3 A). Thus, CRLF2 and mutated IL-7R form a functional TSLP receptor. To test if the presence of mutated IL-7R sensitizes BaF3 cells to TSLP, we treated BaF3 transduced with CRLF2 and either WT IL-7R or IL-7R S185C with increasing doses of TSLP in the absence of IL-3 (Fig. 3 B). Cells expressing CRLF2–IL-7R S185C grew in the absence of cytokines, but also demonstrated higher sensitivity to TSLP. As little as 0.1 ng/ml TSLP doubled the growth rate, but had no effect on BaF3 cells transduced with CRLF2–IL-7R WT. Similarly, cells transduced with IL-7R InsPPCL also responded to TSLP, in addition to marked self-activation of this allele (Fig. S3).

Loss-of-function mutations in IL-7R cause severe combined immunodeficiency characterized by the complete absence of T lymphocytes and the presence of B and NK cells (Puel et al., 1998). Both B- and T-ALLs express IL-7R and have been reported to respond to IL-7 (Touw et al., 1990). We have therefore hypothesized that gain-of-function mutations in IL-7R may also be detected in T-ALL. Accordingly, we screened 295 diagnostic childhood T-ALL samples treated on prospective BFM protocols (Kox et al., 2010) for *IL7R* mutations and identified 30 somatic mutations (10.5%). All the mutations were at the transmembrane domain encoded

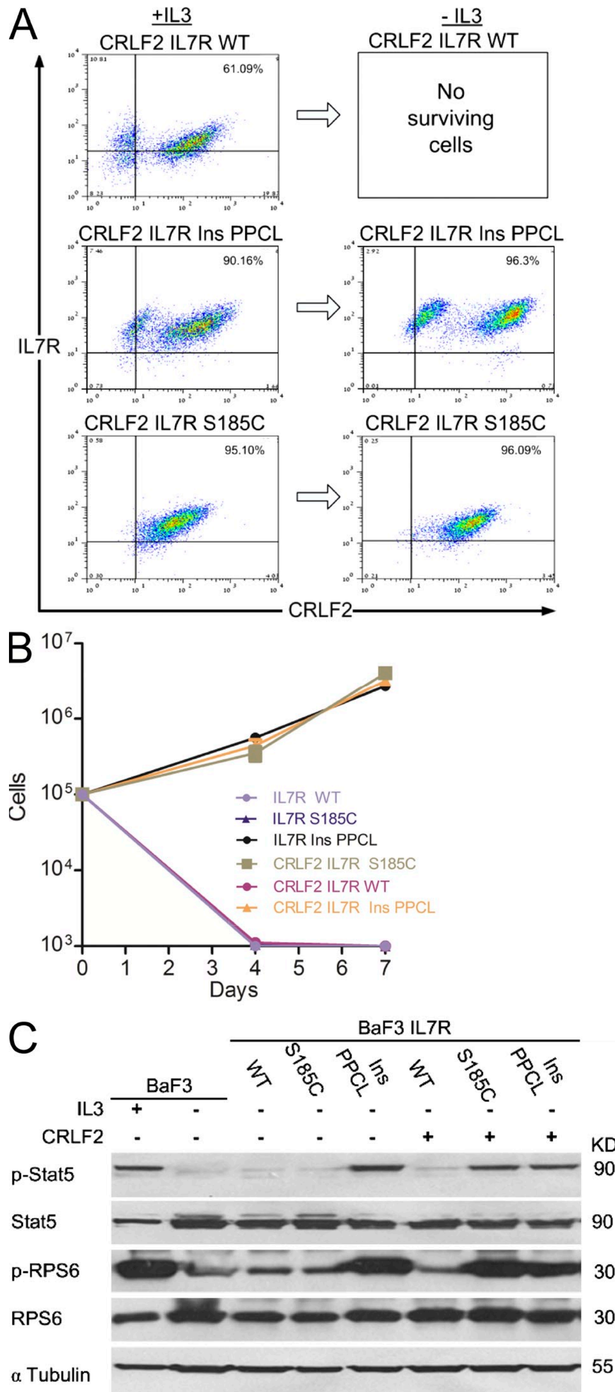

Figure 2. *IL7R* mutations are gain-of-function mutations. (A) FACS analysis of BaF3 cells stably transduced with CRLF2 and either IL-7R WT, IL-7R S185C, or IL-7R InsPPCL in the presence of IL-3. The same cells were then grown without IL-3 for 1 wk and then analyzed again. (B) Cytokine withdrawal assay of BaF3 and BaF3-CRLF2 cells transduced with either IL-7R WT, IL-7R S185C, or IL-7R InsPPCL. Error bars represent SE. (C) Constitutive phosphorylation of Stat5 and ribosomal protein S6 (RPS6) in BaF3 and BaF3-CRLF2 cells expressing IL-7R mutants, after 5 h of cytokine deprivation. IL-3+ indicates cells harvested after 5 h of IL-3 deprivation followed by 20 min of IL-3 stimulation. All experiments were repeated four times.

by exon 6, and all but one were in-frame insertions and deletions (Table II); 27 of the 30 cases included an insertion of cysteine. The patients with mutations were younger and tended to have higher white blood cells counts at diagnosis (Table S1). There was no association between the presence of the mutations and response to therapy, and a similar proportion (10%) relapsed. Notch mutations, which hallmark ~50% of T-ALLs (Clappier et al., 2010; Kox et al., 2010), were detected in 64% of the *IL7R*-mutated leukemias compared with 48% of the T-ALL without *IL7R* mutations (P = 0.1, χ^2 test).

We and others (Hertzberg et al., 2010; Yoda et al., 2010) have recently reported an activating F232C mutation in CRLF2 that introduces cysteine in the juxtamembrane domain. All but three of the mutations observed in IL-7R included the addition of cysteine. To study whether the gain-of-function of the mutated IL-7R could be attributed to the addition of cysteine, we replaced the cysteines in the S815C and in the IL-7R InsPPCL alleles with glycine. These newly mutated receptors were expressed at the cell surface of transduced BaF3 cells (Fig. 4 A). Elimination of the cysteine abrogated the cytokine-independent growth (Fig. 4 B) and the constitutive phosphorylation of Stat5 (Fig. 4 C). Response to TSLP was not altered, confirming that "cysteine-lacking" mutated IL-7R formed a functional TSLP receptor with CRLF2 (Fig. 4 C). These results suggest that the presence of cysteine is critical for the gain-of-function phenotype both in the context of insertion of additional amino acids and as a point mutation.

The experimental insertion of cysteine into the transmembrane domain of the erythropoietin receptor (Constantinescu et al., 2001; Lu et al., 2006) activated the receptor by causing ligand-independent receptor dimerization. Indeed, we observed marked homodimerization of the IL-7R InsPPCL in protein analysis under nonreducing conditions (Fig. 4 D). Interestingly, CRLF2 was not present in these dimers, which is consistent with the observation that this mutant protein does not require CRLF2 for its activation and with the presence of these mutations in T-ALLs in which CRLF2 is not highly expressed. Our current and recent observations (Chapiro et al., 2010; Hertzberg et al., 2010; Yoda et al., 2010) of mutations in IL-7R and CRLF2, respectively, suggest that the addition of cysteine to the juxtamembranous domain is a general mechanism for mutational activation of type I cytokine receptors in leukemias.

These discoveries are a prime example of the interconnection of development and leukemia (Izraeli, 2004). Although loss-of-function mutations in IL-7R perturb lymphoid development, the novel gain-of-function mutations described here are associated with both BCP- and T-ALLs. Activating mutations of IL-7R are present in leukemias of the same lineage, for which it is developmentally required (T cells), or in B cell precursor leukemias that aberrantly express a T cell and monocytic receptor (CRLF2). The broad significance of this pathway in leukemia is further suggested by the aberrant deregulation of CRLF2–JAK–STAT pathway in BCP-ALL

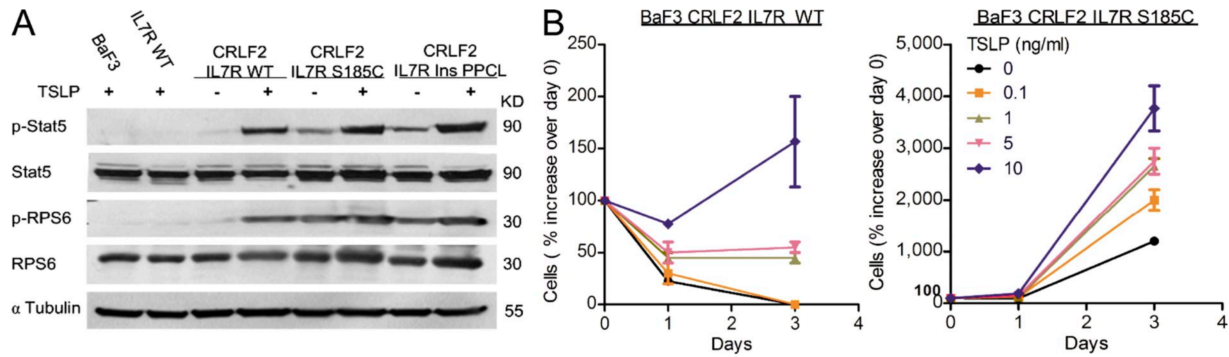

Figure 3. ***CRLF2*** **and mutated** ***IL7R*** **form a functional TSLP receptor and sensitize cells to TSLP.** (A) BaF3 cells expressing CRLF2 and either WT or mutated IL-7R were starved of IL-3 for 5 h, and then treated or not with 100 ng/ml TSLP where indicated for 25 min. (B) BaF3-CRLF2 cells transduced with either WT IL-7R or IL-7R S185C were treated with increasing doses of TSLP in the absence of IL-3. The number of cells was normalized to day 0 (therefore relative growth was measured). Experiments were performed three times. Error bars indicate SE.

(Bercovich et al., 2008; Constantinescu et al., 2008; Kearney et al., 2009; Mullighan et al., 2009; Russell et al., 2009; Hertzberg et al., 2010; Yoda et al., 2010) and by these newly described *IL7R* mutations and the previously reported *JAK1* activating mutations in T-ALL (Flex et al., 2008; Hornakova et al., 2010). Similar to mutational activations of other growth factor receptors in cancer (Gazdar, 2009), our observations have potential implications for targeted therapy.

Table II. *IL7R* mutations in T-ALL

ID	*IL7R* mutation DNA	IL-7R mutation protein
T1	c.816 Ins 15 TTTTGTCGGAAGGAC	243 Ins F**C**RKD
T2	c.815 Ins 7 GAGATGC Del 1 A	243 Ins R**C**
T3	c.818Ins 10 CGTGCCCCCT Del 4 TAAC	243 Ins P**C**PL
T4	c.820 Ins 21 TGCCCGAGCAAGATTGCCCCA + point mutation c826 G->C	244 Ins MPEQD**C**P +S246T
T5	c.798 Ins 11 CCTCCTGGTGC Del 17 AGATGGATCCTATCTTA	237 Ins ASW**C**
T6	c.814 Ins 6 GCCCCC Del 6 TACTAA	242 Ins **C**PP
T7	c.817 Ins 11 GCTGCCCGTCC Del 2 TA	243 Ins R**C**PS
T8	c.815 Ins 16 CGACTGTATTGGGGGTC Del 1 A	242 Ins FD**C**IGV
T9	c.822 Ins 10 CACCGTGGGT Del 4 ATCA	245 Ins HRG**C**
T10	c.817 Ins 12 CCCTCTGTTCGG Del 3 TAA	243 Ins PL**C**SA
T11	c.849 Ins 9 GAGAGGCCG	254 Ins GEA
T12	c.817 Ins 19 CCATTTATCGGTGTGTCCT Del 4 TAAC	243 Ins PIYR**C**VL
T13	c.799 Ins 11 CCTCCTGGTGC Del 17 AGATGGATCCTATCTTA	237 Ins ASW**C**
T14	c.816 Ins 7 TGAGTGT Del 1 A	242 Ins FE**C**
T15	c.815 Ins 11 TACCTGCCCGT Del 5 ACTAA	242 Ins FT**C**PS
T16	c.816 Ins 11 TGCCCCTCTCC Del 2 CT	243 Ins **C**PSP
T17	c.850 Ins 6 AAAAAG Del 3 CTC	254 Ins EKV
T18	c.823 Ins 12 GTCATCAGCCCT Del 3 TCA	245 Ins SHQP**C**
T19	c.838 Ins 24 GTTCAACCATCAGCATTTTGAGTT	250 Ins **C**STISILS
T20	c.832 Ins 7 GTCAAAG Del 13 TGAGTTTTTTCTC	248 Ins **C**Q
T21	c. 814 Ins 14 GTGGTATAAGGGAA Del 5 TACTA	242 Ins **C**GIREI
T22	c. 847 T>G	V253G
T23	c. 817 Ins 6 GGCTGT	243 Ins R**C**
T24	c.817 Ins 6 GCTGTA	244 Ins G**C**
T25	c.809 Ins 13 GTGCCGTCCCCAT Del 7 TATCTTA	241 Ins **C**RPH
T26	c.816 Ins 7 GGCTGTA Del 1 C	243 Ins G**C**I
T27	c.821 Ins 12 GAGGCCTTGTGG	245 Ins RP**C**G
T28	c.821 Ins 15 ACTTCCCTGCGTCTAC	245 Ins LP**C**VT
T29	c.814 Ins 10 GCTGGATGAA Del 1 T	242 Ins **C**WMK
T30	c.820 Ins 13 AGAAATGCACAAA Del 1 C	244 Ins KK**C**TN

Del, Deletion; Ins, Insertion. The amino acid cysteine included in 27 of 30 mutations is shown in bold.

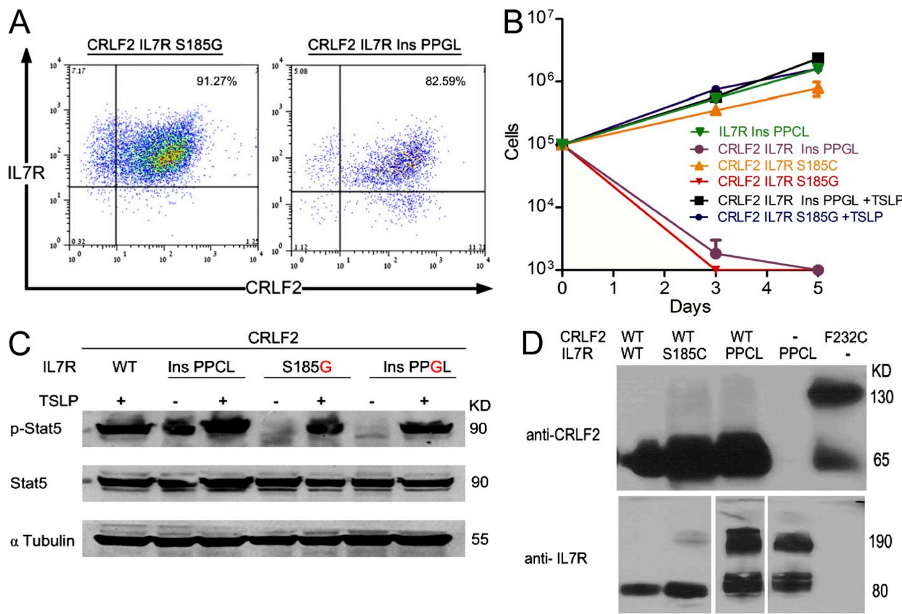

Figure 4. Functional significance of the cysteine residue in mutated *IL7R*. (A) FACS analysis of BaF3-CRLF2 cells expressing either S185G or IL-7R InsPPGL. In these constructs, the cysteine was mutated to glycine. (B) Growth assay of BaF3 and BaF3-CRLF2 cells transduced with IL-7R WT, IL-7R S185G, or IL-7R InsPPGL in the presence or absence of 30 ng/ml TSLP and the absence of IL-3. Error bars indicate SE. (C) BaF3 and BaF3-CRLF2 cells expressing indicated IL-7R mutants were incubated without IL-3 for 5 h. Where indicated, 100 ng/ml TSLP was added for 25 min. (D) Homodimerization of IL-7R InsPPCL mutant under nonreducing conditions. BaF3 cells that stably express CRLF2 with IL-7R (WT or mutated), IL-7R InsPPCL alone, or CRLF2 F232C (that has been shown previously to homodimerize; Yoda et al., 2010), were grown in the presence of IL-3. Protein lysates were separated by gel electrophoresis in the presence of nonreducing conditions and immunoblotted with an anti-CRLF2 or anti–IL-7R antibody. All experiments were performed three times.

MATERIALS AND METHODS

Patient samples. DNA and RNA were derived from leukemic bone marrow of children with ALL enrolled in a prospective BFM (Berlin Frankfurt Munster) and AIEOP (Associazione Italiana Ematologia Oncologia Pediatrica Group) ALL 2000 study (Conter et al., 2010). The T-ALL and the DS-ALL cohorts were previously described (Hertzberg et al., 2010; Kox et al., 2010). All specimens were collected with informed consent and approval of local and national ethic committees. Samples were anonymized for the study. The study was approved by the Israeli Health Ministry Ethic committee, approval # 920070771.

CRLF2 analysis. CRLF2 levels were analyzed by the TaqMan Gene Expression Assay Hs00913509_s1 (Applied Biosystems), as we have previously reported (Cario et al., 2010; Hertzberg et al., 2010). The presence of the fusion transcript *P2RY8-CRLF2* was analyzed by RT-PCR, as we have previously reported (Cario et al., 2010; Hertzberg et al., 2010), using primers designed in the first exon of *P2RY8* (5′-GGACAGATGGAACTGGAAGG-3′) and the third exon of *CRLF2* (5′-GTCCCATTCCTGATGGAGAA-3′). PCR product was ~511 bp. Immunoglobulin heavy chain locus (IGHα) translocations were identified by fluorescence in situ hybridization (FISH) on interphase nuclei using the Vysis LSI *IGH*α Dual Color Break-Apart Rearrangement Probe (Abbott Molecular). The nuclei were counterstained with DAPI (4′, 6-diamino-2-phenylindol). Results were recorded using a fluorescence microscope (DMRB; Leica) fitted with a 100×/1.30 oil objective, charge-coupled device camera, and digital imaging software from MetaSystems (Isis FISH imaging system).

For the purpose of this study, the CRLF2-positive group consisted of BCP-ALL specimens that were either positive for the *P2RY8-CRLF2* fusion or to the IGHα CRLF2 translocation or had CRLF2 RNA expression levels >20 fold of the median value of CRLF2 expression of 555 unselected BCP-ALLs (Cario et al., 2010). The CRLF2-negative group consisted of BCP-ALL specimens negative for the genomic rearrangements and with expression level at the median and below. *JAK2* and *CRLF2* mutations were identified as previously described (Hertzberg et al., 2010)

***IL7R* mutation analysis.** We used intronic primers of human *IL7R* sequence (available from GenBank/EMBL/DDBJ under accession no. NM_002185.2; Table S2) to amplify exons 1–8 of the gene with PCR. Fast start Taq DNA polymerase (Roche) was used with the following thermal cycling conditions: 1 cycle of 94°C for 5 min, followed by 5 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C 30 s, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C 30 s, followed by 1 cycle of 72°C for 7 min. We then analyzed the fragments by denaturing high-performance liquid chromatography as described before (Bercovich et al., 2008; Hertzberg et al., 2010).

Fragments with abnormal chromatography patterns were sequenced directly by the primer used for PCR, or cloned into TA vector pCR2.1 (Invitrogen). DNA from plasmid clones was extracted by AccuPrep Spin Miniprep kit (Bioneer) and sequenced.

Site-directed mutagenesis. The human *IL7R* cloned into the MSCVires-GFP retrovirus (encoding GFP) was used as a template for the generation of mutations by site-directed mutagenesis (QuikChange II XL; Stratagene). The plasmid sequences were verified by sequencing.

BaF3 assays. Parental BaF3 cells and those expressing human *CRLF2* receptor (BaF3/CRLF2; Hertzberg et al., 2010) were transduced with the retroviruses containing WT or mutant *IL7R*. Transduced cells were sorted by flow cytometer 2–4 d later. PE anti–human CRLF2 (322806; BioLegend) and Alexa Fluor 647 anti–human CD127 (IL-7R, 317606; BioLegend) antibodies were used. Cytokine withdrawal assays were performed as previously described (Hertzberg et al., 2010)

Western blotting. For Western blot analyses, 10% polyacrylamide linear gels were used. BaF3 cells were harvested after 5 h of IL-3 deprivation, and then stimulated with either 10 ng/ml IL-3 or 100 ng/ml TSLP for 25 min. The antibodies used were anti–phospho-STAT5 Tyr 694 (Epitomics), anti-STAT5, anti-S6 ribosomal protein and anti-phospho-S6 ribosomal protein Ser 235/6 (Cell Signaling Technology).

For Western blot analyses of disulfide-bonded dimmers, 8% polyacrylamide linear gels were used. For nonreducing conditions, proteins were boiled in SDS buffer without 2-mercaptoethanol. Antibodies used were anti–human IL-7R (R&D Systems) and anti–human CRLF2 (BioLegend).

Online supplemental material. Fig. S1 shows expression of mutated *IL7R*. Fig. S2 shows FACS analysis of parental BaF3 cells in the presence of IL-3. Fig. S3 shows *CRLF2* and *IL7R* Ins PPCL respond to TSLP. Table S1 shows the clinical characteristics of T-ALL patients with *IL7R*

mutations. Table S2 shows primers used for genomic amplification of *IL7R* exons. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20110580/DC1.

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