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Role of casein kinase 1A1 in the biology and targeted therapy of del(5q) MDS

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Summary

The Casein kinase 1A1 gene (*CSNK1A1)* is a putative tumor suppressor gene located in the common deleted region for del(5q) myelodysplastic syndrome (MDS). We generated a murine model with conditional inactivation of *Csnk1a1* and found that *Csnk1a1* haploinsufficiency

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R.K.S, D.H., M.J. and B.L.E. designed experiments. R.K.S, D.H., A.M.L, L.P.C., M.E.M., A.M. and R.K. performed experiments and analyzed data. V.A., M.M., R.B. and F.S. collected patient samples and clinical information, performed whole exome sequencing, validation by Sanger sequencing and analyzed these data. R.K.S and B.L.E. wrote the manuscript. All authors provided critical review of the manuscript.

induces hematopoietic stem cell expansion and a competitive repopulation advantage whereas homozygous deletion induces hematopoietic stem cell failure. Based on this finding, we found that heterozygous inactivation of *Csnk1a1* sensitizes cells to a *CSNK1* inhibitor relative to cells with two intact alleles. In addition, we identified recurrent somatic mutations in *CSNK1A1* on the non-deleted allele of patients with del(5q) MDS. These studies demonstrate that *CSNK1A1* plays a central role in the biology of del(5q) MDS and is a promising therapeutic target.

Introduction

Deletions of chromosome 5q are the most common cytogenetic abnormalities in MDS, and patients with isolated del(5q) have a distinct clinical phenotype (Ebert, 2011; Haase et al., 2007; Hasserjian, 2008). To date, no genes within the common deleted regions (CDR) have been found to undergo homozygous inactivation, copy-neutral loss of heterozygosity, or recurrent mutation (Gondek et al., 2008; Graubert et al., 2009; Heinrichs et al., 2009; Jerez et al., 2012; Mallo et al., 2013). Functional studies have revealed individual genes that contribute cooperatively to the clinical phenotype through genetic haploinsufficiency (Boultwood et al., 2010; Chen et al., 2011; Ebert, 2011; Kumar et al., 2011; Lane et al., 2010; Starczynowski et al., 2010). Heterozygous loss of the *RPS14* gene, for example, has been linked to impaired erythropoiesis via p53 activation (Dutt et al., 2011; Ebert et al., 2008). While several 5q genes have been reported to alter hematopoietic stem cell function, the mechanism of clonal dominance of del(5q) cells remains a critical unsolved question (Joslin et al., 2007; Lane et al., 2010; Min et al., 2008; Wang et al., 2010a).

CSNK1A1 encodes casein kinase 1α (CK1α), a serine/threonine kinase, and is located in the distal common deleted region (5q32) in del(5q) MDS. In a careful study of gene expression in $CD34^+$ cells from a large cohort of del(5q) and other MDS cases, *CSNK1A1* was one of the few genes in the del(5q) common deleted region that has approximately 50% normal expression (Boultwood et al., 2007). Recent studies demonstrated that *CSNK1A1* is a tumor suppressor gene in colon cancer and melanoma controlling proliferation by its function as a central regulator of β-catenin activity (Elyada et al., 2011; Sinnberg et al., 2010). In hematopoiesis, stem and progenitor cells respond in a graded fashion to canonical Wnt/β-catenin signaling (Luis et al., 2011). Constitutive activation of β-catenin has been reported to increase HSC numbers followed by apoptosis, HSC depletion, and bone marrow failure (Kirstetter et al., 2006; Scheller et al., 2006). In contrast, less profound activation is associated with HSC expansion with enhanced repopulation potential (Trowbridge et al., 2006). APC, like CK1α, is a member of the β-catenin destruction complex, and is inactivated in approximately 95% of cases with del(5q) MDS. Mice with heterozygous deletion of *Apc* (Wang et al., 2010a) or heterozygous for the Apc^{Min} allele (Lane et al., 2010) have increased repopulation potential in primary bone marrow transplants, but decreased repopulation potential of secondary transplants due to loss of HSC quiescence.

We sought to explore whether haploinsufficiency or mutation of *Csnk1a1* contributes to the biology of del(5q) MDS. In addition, given evidence that *Csnk1a1* is selectively essential for murine MLL-AF9 leukemia cells relative to normal hematopoietic cells (Jaras et al., 2014), we investigated whether CK1α is a therapeutic target in del(5q) MDS.

Results

Csnk1a1 is required for adult murine hematopoiesis

To explore the role of *Csnk1a1* on hematopoietic stem cell (HSC) function, we generated a mouse model in which *Csnk1a1* exon 3, essential for CK1α kinase function (Bidere et al., 2009), is flanked by loxP sites. Following crosses to *Mx1Cre* transgenic mice, we induced *Csnk1a1* excision in hematopoietic cells by poly(I:C), and confirmed decreased mRNA and protein expression (Figure 1A and S1A).

We first examined whether *Csnk1a1* plays a critical role in hematopoiesis. Homozygous deletion of *Csnk1a1* in the hematopoietic system (*Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$) resulted in rapid lethality 5–17 days after gene excision, accompanied by a significant decrease in all peripheral blood counts and histologic evidence of fulminant bone marrow failure with evidence of ischemia in multiple organs (Figures 1B, C, D, E and S1B).

We next examined whether the observed hematologic abnormalities were associated with changes in hematopoietic stem and progenitor cells. Ten days after *Csnk1a1* excision, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ mice had a highly significant reduction of HSC (LSK, lin^{low}Sca1^{+}ckit^{+}) including long-term (LT, lin^{low}Sca1^{+}ckit^{+}CD150^{+}CD48^{-}), short-term (ST, lin^{low}Sca1^{+}ckit^{+}CD150^{-}CD48^{-}) HSC and multipotent progenitor cells (MPP, lin^{low}Sca1^{+}ckit^{+}CD150^{-}CD48^{+}) indicating that *Csnk1a1* is essential for HSC survival (Figure 1F and S1C).

CK1α is a major regulator of p53 activity, so we investigated whether *Csnk1a1* ablation activates p53 in the bone marrow (Elyada et al., 2011; Wu et al., 2012). Homozygous, but not heterozygous *Csnk1a1* deletion caused accumulation of p53 as well as p21, a p53 target, demonstrating that p53 is both present and active (Figure 1G). Consistent with this finding, we found that only complete ablation of *Csnk1a1* led to significant induction of early and late apoptosis (Figure 1H and S1D). *Csnk1a1*-ablated HSC exited quiescence and entered the cell cycle, with a marked decrease in the number of *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ HSC (LSK) in G0 and a significant increase in S/G2/M compared to *Mx1Cre*$^{+}$ controls (Figure 1I and S1E–F).

Csnk1a1 loss induces increased β-catenin levels in both hematopoietic and stromal cells

CK1α is a critical regulator of β-catenin (Cheong and Virshup, 2011). In our murine model, heterozygous and homozygous knockout of *Csnk1a1* induced strong nuclear accumulation of β-catenin (Figure 1J). In the heterozygous knockout bone marrow, positive staining was predominantly in hematopoietic cells proximal to endothelial and endosteal cells, while in the homozygous knockout bone marrow, β-catenin nuclear accumulation was observed in nearly all cell types, highlighting a graded β-catenin activation by *Csnk1a1* gene dosage.

In addition, we observed a striking accumulation of β-catenin in the bone marrow stroma cells of heterozygous and homozygous *Csnk1a1* knockout mice, consistent with the expression of *Mx1Cre* in bone marrow stroma (Walkley et al., 2007). We validated this finding in mesenchymal stroma cells (MSC) isolated from endosteal bone (Zhu et al., 2010) and confirmed *Csnk1a1* excision in the stroma (Figure S1G). We found strong β-catenin

expression in MSC from heterozygous *Csnk1a1* knockout mice, and even more pronounced expression in homozygous knockout mice (Figure S1H). In in vitro long-term culture initiating cell assays, both *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ and *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ MSC had significantly impaired hematopoiesis-supporting capacity. Inactivation of β-catenin rescued the effect of *Csnk1a1* loss in stromal cells (Figure S1I). The hematopoietic effects of *Csnk1a1* haploinsufficiency that we found in vitro were also observed in vivo. Eight weeks after pIpC treatment, we observed a significant reduction in bone marrow cellularity and in the percentage of LT- and ST-HSC in *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ mice compared to *Mx1Cre*$^{+}$ controls (Figure S1J–M). Consistent with this finding, the survival of *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ primary mice was significantly impaired (Figure 1B). After 15 months *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ mice developed pancytopenia, a significant decrease in the LT-HSC and ST-HSC, and a near complete loss of myeloid progenitor cells (Figure S1N–V). These results are comparable to the recently described consequences of constitutively active β-catenin in osteoblasts (Kode et al., 2014), though we did not observe any evidence of malignant transformation in *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ mice at 15 months.

Cell intrinsic *Csnk1a1* ablation leads to bone marrow failure

Having observed that *Csnk1a1* excision in primary *Mx1Cre*$^{+}$ mice has striking effects on hematopoiesis, and given the cell-extrinsic effects of stromal β-catenin activation on hematopoietic cells (Kode et al., 2014; Lane et al., 2010; Stoddart et al., 2013; Wang et al., 2010a), we examined the cell-intrinsic effects of *Csnk1a1* inactivation in hematopoietic cells using bone marrow transplantation into wild-type recipient mice. We transplanted whole bone marrow cells from *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ or *Mx1Cre*$^{+}$ mice (CD45.2^{+}) into lethally irradiated WT recipient mice (CD45.1^{+}). Prior to induction of *Csnk1a1* excision, 4 weeks after transplantation, more than 90% of peripheral blood cells in recipient mice were reconstituted with donor-derived CD45.2^{+} cells. All recipient chimeric mice reconstituted with *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ cells became moribund with bone marrow failure 8–14 days after *Csnk1a1* excision (Figure 2A and S2A–C). Flow cytometric analysis revealed a complete loss of hematopoietic stem and progenitor cells in recipient mice. These studies confirm that a cell intrinsic function of *Csnk1a1* is essential for hematopoiesis.

In striking contrast to mice transplanted with *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ cells, mice transplanted with *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ had no change in survival compared to *Mx1Cre*$^{+}$ control mice (Figure S2A). Transplanted *Csnk1a1* haploinsufficient hematopoietic cells fully reconstituted the bone marrow, resulting in a normal to hypercellular marrow, a normal hemoglobin, and significantly elevated white blood cell counts with lymphocytosis (Figures 2B–D). The lymphocytosis was caused by an increase in T-cells, consistent with reports demonstrating that moderate Wnt-activation promotes T-cell differentiation (Luis et al., 2012; Luis et al., 2011). The percentage of Gr1^{+}CD11b^{+} myeloid cells (Figure 2E) and CD19^{+} B-cells (Figure S2D) was not affected.

Pathological evaluation of the *Csnk1a1* haploinsufficient bone marrow revealed increased and mildly dysplastic hypolobulated (micro) megakaryocytes in atypical locations, reminiscent of the megakaryocyte morphology in del(5q) MDS (Figure 2F and S2E–F). This phenotype was recapitulated in vitro when whole bone marrow cells were cultured in the

presence of 10 ng/ml murine thrombopoietin. Nuclear ploidy analysis of the $CD41^+$ megakaryocytes revealed a shift towards hypoploidy, consistent with hypolobation apparent in cytospins of the cultures (Figure 2G, H). Over time, the mice developed a significantly elevated platelet count (Figure 2I).

Haploinsufficiency of *Csnk1a1* leads to β-catenin activation and cell-intrinsic expansion of hematopoietic stem cells

CSNK1A1 has been reported to be a tumor suppressor gene in solid tumors due to activation of β-catenin (Elyada et al., 2011; Sinnberg et al., 2010). We first examined whether *Csnk1a* haploinsufficiency causes a cell-intrinsic effect on the number and function of HSC in a non-competitive transplantation assay. We found an increase in the percentage of the HSC-enriched LSK compartment, in contrast to the decrease in LSK and LK cells observed in the setting of an abnormal microenvironment in primary $Mx1Cre^+$ animals (Figure 1F and S1M, R). In particular, the proportion of LT-HSC was significantly elevated (Figure 3A).

To analyze whether HSC expansion might be due to exit from quiescence and enhanced HSC proliferation, we performed cell cycle analysis on hematopoietic stem and progenitor cells. In comparison to $CD45.2^+$ $Mx1Cre^+$ control baseline hematopoiesis, $CD45.2^+$ LSK cells and LT-HSC from $Csnk1a1^{-/-}Mx1Cre^+$ cells had a significantly lower percentage of cells in the quiescent G0 fraction (in LT-HSC not LSK) and a significantly higher percentage of cells in the cycling G1 fraction (Figure 3B) and in the S-phase as seen by BrdU incorporation (Figure S3A), consistent with exit from quiescence.

We next examined whether *Csnk1a1* heterozygous hematopoietic stem cells have altered β-catenin or cyclin D1 activity, as these pathways could contribute to decreased quiescence. $Csnk1a1^{-/+}Mx1Cre^+$ hematopoietic cells, transplanted into WT mice, had increased nuclear β-catenin accumulation by immunohistochemistry (Figure S3B) and by intracellular flow cytometry (Figure 3C–E). We found increased β-catenin in the stem cell enriched LSK fraction. β-catenin accumulation in HSC was accompanied by significantly increased expression of cyclin D1, a major regulator of cell cycle progression, corroborating the G1-phase progression in the cell cycle of *Csnk1a1* haploinsufficient cells. In the lineage-positive fraction, the differences in β-catenin were not apparent. These experiments demonstrate that heterozygous *Csnk1a1* inactivation is associated with increased levels of β-catenin in the hematopoietic stem cell.

As heterozygous deletion of *APC* occurs in approximately 95% of MDS cases and APC and CK1α are both negative regulators of β-catenin, we analyzed the combinatorial effect of *Csnk1a1* and *Apc* on β-catenin levels and hematopoietic stem cell expansion (Figure S3F). Compound heterozygous ($Csnk1a1^{-/+}Apc^{-/+}Mx1Cre^+$) hematopoietic cells were transplanted into lethally irradiated mice and analyzed over a period of 52 weeks. Compound heterozygous inactivation of *Csnk1a1* and *Apc* resulted in significantly increased LT-HSCs, increased β-catenin levels, and increased activation of the cell cycle in hematopoietic stem cells in long-term transplants (Figure S3G–J). In aggregate, our data highlight a central role for β-catenin in the pathophysiology of del(5q) MDS.

Csnk1a1 haploinsufficient HSCs have increased self-renewal ability in vivo

As *Csnk1a1* haploinsufficiency leads to a significant increase in cycling LT-HSCs, we examined the functional capacity of *Csnk1a1* haploinsufficient cells in a competitive repopulation assay. Four weeks after transplantation, mice were treated with poly(I:C) to induce *Csnk1a1* deletion. *Csnk1a1* haploinsufficient cells out-competed WT cells, while *Mx1Cre*$^+$ control cells were stable over time, and cells with homozygous *Csnk1a1* inactivation were rapidly depleted (Figure 4A).

To determine the long-term repopulating potential of *Csnk1a1* haploinsufficient bone marrow, whole bone marrow cells from the primary recipients were injected into lethally irradiated secondary and tertiary recipients. *Csnk1a1* haploinsufficient bone marrow cells had a significantly impaired response to the stress of transplantation, resulting in significantly lower numbers of CD45.2$^+$ donors cells in the peripheral blood compared to *Mx1Cre*$^+$ controls each 4 weeks after secondary and tertiary transplantation. However, 16 weeks after each round of transplantation, *Csnk1a1* haploinsufficient cells recovered and again out-competed the control cells (Figure 4A).

Having observed a competitive advantage for *Csnk1a1* haploinsufficient bone marrow evaluated in the peripheral blood, we next evaluated hematopoietic stem and progenitor cells in the setting of competitive repopulation (Figure 4B–E). At both 16 weeks following the primary transplant and 16 weeks following the secondary transplant, *Csnk1a1* haploinsufficient cells were significantly more abundant than their wild type counterparts in the percentage of LSK cells and downstream myeloid progenitor cells, Gr1$^+$CD11b$^+$ myeloid cells, and CD3$^+$ T-cells in the bone marrow.

Csnk1a1 haploinsufficiency sensitizes cells to casein kinase 1 inhibition

Having demonstrated a selective advantage for cells with heterozygous *Csnk1a1* inactivation, and a severe disadvantage for cells with homozygous *Csnk1a1* inactivation, we postulated that *Csnk1a1* haploinsufficiency might sensitize cells to CK1α inhibition. Partial inhibition of CK1α would be expected to cause wild-type cells to have a phenotype similar to haploinsufficient cells, while CK1α inhibition in cells that already have one allele inactivated would approach closer to complete ablation of CK1α activity, thereby establishing a therapeutic window for CK1α inhibition in del(5q) MDS cells. We tested this hypothesis using D4476, a selective small molecule inhibitor of CK1 (Rena et al., 2004). Since D4476 has a short half-life in vivo, we treated purified myeloid progenitors from *Csnk1a1* haploinsufficient cells and *Mx1Cre*$^+$ controls with D4476 in vitro. D4476 significantly decreased viability and increased apoptosis in *Csnk1a1* haploinsufficient cells relative to *Mx1Cre*$^+$ controls at a range of concentrations, consistent with a therapeutic window for targeting *Csnk1a1* haploinsufficient cells (Figure 5A).

To assess the relative effect of D4476 treatment on HSC and progenitor cell function in vivo, we performed a competitive repopulation experiment following ex vivo exposure to D4476. Purified LSK cells from *Csnk1a1*$^{-/+}$*Mx1Cre*$^+$ (CD45.2) and WT CD45.1 mice were mixed in a 1:1 ratio and treated ex vivo with either D4476 or DMSO control for 48 hours, followed by injection of the cells into lethally irradiated mice. Following DMSO treatment,

Csnk1a1 haploinsufficient cells out-competed the wild-type controls, as assessed by peripheral blood chimerism. In contrast, following treatment with D4476, *Csnk1a1* haploinsufficient cells were selectively depleted. Similarly, D4476 caused a selective depletion of *Csnk1a1* haploinsufficient stem and progenitor cells in the bone marrow (Figure 5B–C) and reduced *Csnk1a1* haploinsufficient hematopoietic stem and progenitor cells in colony-forming unit assays (Figure S4A).

To examine if partial, systemic inhibition of CK1α would be tolerated in a therapeutic approach targeting haploinsufficiency, we analyzed the effects of global heterozygous *Csnk1a1* inactivation. *Csnk1a1*$^{-/+}$*EIIaCre*$^{+}$ mice, in which heterozygous deletion of *Csnk1a1* is induced in all tissues, were born in normal Mendelian ratios without apparent malformations. Histopathological analysis at 6 and 10 months of age revealed structural integrity of organs, and blood counts were normal and stable over this period of time (Figure S4B–D). In aggregate, these data indicate that *Csnk1a1* inhibition is an attractive therapeutic approach for the selective targeting of *Csnk1a1* haploinsufficient cells, such as MDS cells with del(5q).

Identification of recurrent somatic *CSNK1A1* mutations in patients with del(5q) MDS

In parallel with our functional studies, we performed whole-exome sequencing on MDS samples to identify genetic drivers of del(5q) MDS: genes that are selectively mutated in del(5q) MDS, or genes within the del(5q) CDRs that are recurrently somatically mutated in MDS cases without del(5q). We performed whole-exome sequencing on paired samples (MDS-derived bone marrow sample and matched normal CD3^{+} cells) of 21 cases: 19 del(5q) and 2 with normal karyotypes (Table S1). We identified two cases with somatic mutations in *CSNK1A1*, both in untreated cases with del(5q) with wild-type *TP53* (Table 1). Both mutations caused the same amino acid change, E98K (Figure 6A). The mutations were confirmed to be present and somatic by Sanger sequencing (Figure 6A). Only a fraction (75% in patient 1 and 42% in patient 2) of the non-deleted *CSNK1A1* allele were mutated. By SNP array analysis (Figure S5A), the percentage of the del(5q) MDS clone was 70–80% in patient 1 and 90–100% in patient 2. These data indicate that deletion of chromosome 5q occurred first, and that the *CSNK1A1* mutation occurred on the remaining allele of chromosome 5q. The mutation was identified in only less than 5% of the matched control samples from T cells. We analyzed an additional set of 22 MDS samples with isolated del(5q) and found one additional mutation, also altering the same codon (E98V). We examined published MDS genome-sequencing data and found one *CSNK1A1* mutation, D140A, in a case with MDS and a normal karyotype (Graubert et al., 2012) and *CSNK1A1* D140Y in a patient with del(5q) MDS (Woll et al., 2014). Additional *CSNK1A1* mutations were identified in the literature in other malignancies (Dulak et al., 2013; Sato et al., 2013), two of which are also missense mutations of codon 98, and one of codon 140 (Figure S5B). *CSNK1A1* is therefore a gene with recurrent somatic mutations within a del(5q) CDR in MDS.

We tested the function of the *CSNK1A1 E98V* mutation by retroviral expression of the mutant cDNA in *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ hematopoietic cells, reflecting the finding of mutations in del(5q) cells without a wild-type allele. Ckit^{+} hematopoietic cells were

transduced with retroviruses expressing a wild type *CSNK1A1* cDNA, the *CSNK1A1 E98V* mutation, or the *CSNK1A1 D136N* cDNA with mutational inactivation of the CK1α kinase activity (Bidere et al., 2009; Davidson et al., 2005; Peters et al., 1999). Four weeks after transplantation of transduced cells into lethally irradiated recipients, we induced excision of both endogenous *Csnk1a1* alleles. Mice transplanted with cells expressing the kinase-dead *CSNK1A1* D136N cDNA died rapidly as expected, as the mutant cDNA was unable to rescue the effect of the *Csnk1a1* ablation (Figure S5C–E). In contrast, cDNA overexpressing *CSNK1A1* and *CSNK1A1* E98V cDNA rescued the HSC ablation in $Csnk1a1^{-/-}Mx1Cre^{+}$ cells (Figure 6B and S5C). After 12 weeks, the bone marrow of the recipient mice was fully reconstituted by cells transduced with either *CSNK1A1* cDNA or *CSNK1A1 E98V* cDNA (Figure 6C). Cells expressing *CSNK1A1* or *CSNK1A1 E98V* reconstituted lineages, as well as stem and progenitor cells (Figures 6C and S5F).

We next examined the cellular consequences of the *CSNK1A1 E98V* mutation. $Csnk1a1^{-/-}Mx1Cre^{+}$ cells transduced with *CSNK1A1 E98V* cDNA, compared to cells expressing the wild-type cDNA, had increased nuclear β-catenin accumulation by immunofluorescence, and higher β-catenin accumulation by intracellular flow cytometry (Figure 6D–F and S5G). While expression of the kinase-dead *CSNK1A1 D136N* cDNA caused increased apoptosis and HSC ablation, the *CSNK1A1 E98V* cDNA did not induce p53 or apoptosis (Figure 6, D, F and S5G, H). Furthermore, bone marrow cells expressing *CSNK1A1 E98V* cDNA had an increased frequency of cells in the G1 phase of the cell cycle, with no change in cells in G0 (Figure 6G). In aggregate, these findings indicate that the codon 98 mutations are not loss-of-function, and do not cause increased p53 activation, but do increase β-catenin activity, providing a potential selective advantage to del(5q) MDS cells.

Having demonstrated that *Csnk1a1* haploinsufficient cells are sensitized to CK1 inhibition, we tested whether *CSNK1A1* E98V-expressing cells in a *Csnk1a1* null background are more sensitive to treatment with a CK1 inhibitor than wild-type or *Csnk1a1* haploinsufficient cells. GFP^{+} *CSNK1A1* E98V and *CSNK1A1* expressing LSK cells and *Csnk1a1* haploinsufficient and wild-type LSK cells were sorted and treated with D4476 (Figure 6H). Treatment of $Csnk1a1^{-/-}Mx1Cre^{+}$ cells transduced with *CSNK1A1 E98V* cDNA were significantly more sensitive to the compound than *Csnk1a1* haploinsufficient cells. Similar results were obtained from a co-culture competition assay in the presence of D4476 (Figure 6I and S5I).

Discussion

Our studies converged on a critical role for CK1α in the pathogenesis of del(5q) MDS. Activation of β-catenin downstream of *Csnk1a1* haploinsufficiency in a murine model, and downstream of *CSNK1A1* mutations in MDS patient samples, provides a potential mechanism of clonal selection. In contrast, homozygous inactivation of *Csnk1a1* is not tolerated due to activation of p53. The sensitivity of hematopoietic cells to *Csnk1a1* gene dosage provides a therapeutic window for targeting CK1α in haploinsufficient cells.

In a previous study, we found *Csnk1a1* to be a therapeutic target in AML, and that D4476 selectively kills leukemic stem cells relative to normal hematopoietic stem and progenitor cells (Jaras et al., 2014). Both the knockdown of *Csnk1a1* using shRNA and the genetically engineered mouse model show that reduction of *Csnk1a1* expression by more than 50% has a negative effect on hematopoietic stem cell expansion and survival. Haploinsufficiency, in contrast, increases the number and function of hematopoietic stem cells.

β-catenin is a major driver of stem cell self-renewal and neoplasia in multiple cellular lineages (Baba et al., 2005; Elyada et al., 2011; Willert et al., 2003; Yeung et al., 2010). Hematopoietic stem cells have a graded response to β-catenin, with modest levels leading to increased stem cell self-renewal (Baba et al., 2005), and more marked induction leading to stem cell exhaustion (Albuquerque et al., 2002; Kirstetter et al., 2006; Lane et al., 2010; Luis et al., 2011). Forced expression of β-catenin, in combination with HOXA9 and MEIS1, induces leukemia in progenitor cells (Wang et al., 2010b), and β-catenin is essential for leukemia cells driven by the MLL-AF9 oncogene (Miller et al., 2013). Histopathological studies have found nuclear, non-phosphorylated β-catenin expression in bone marrow specimen from de novo AML and MDS patients to be a predictor for clinical outcome, and these studies suggested an association between nuclear β-catenin expression and del(5q) MDS, though the number of samples studied was too small to be conclusive (Xu et al., 2008). CK1α is a member of the β-catenin destruction complex and is therefore a known, central regulator of β-catenin activity (Cheong and Virshup, 2011). In our studies, *Csnk1a1* haploinsufficiency conferred to increased intrinsic self-renewal of HSC, with associated nuclear β-catenin accumulation, cyclin D1 induction, and exit from quiescence in LT-HSCs.

Increased LT-HSC proliferation and expansion was a cell-intrinsic effect in our study. Inactivation of *Csnk1a1* in stromal cells in our model caused stromal β-catenin levels to increase, with consequent effects on hematopoiesis, including pancytopenia and hematopoietic stem and progenitor cell depletion. This observation is consistent with recent studies demonstrating that β-catenin accumulation in the stroma negatively regulates HSC maintenance and might also contribute to leukaemogenesis (Kode et al., 2014; Lane et al., 2010).

APC, another member of the β-catenin destruction complex, is also deleted in the vast majority of del(5q) MDS cases. Hematopoietic cells with *Apc* haploinsufficiency have been shown to have enhanced repopulation potential, indicating a cell intrinsic gain of function in the LT-HSC population. However, in contrast to *Csnk1a1* haploinsufficiency, *Apc* haploinsufficient bone marrow was unable to repopulate secondary recipients due to loss of the quiescent HSC population (Lane et al., 2010; Wang et al., 2010a). Different levels of Wnt activation may explain these findings. Similarly, deletions of *Csnk1a1* and of *Apc* in the gut have significantly different effects. While *Csnk1a1* deletion led to robust activation of Wnt target genes and proliferation without invasion, *Apc* deletion induced immediate dysplastic transformation and rapid death (Elyada et al., 2011). CK1α has many phosphorylation targets that could alter stem cell function (Bidere et al., 2009; Elyada et al., 2011; Wu et al., 2012). As has been postulated previously, it is possible that CK1α inactivation restrains hyperactive Wnt signaling through mechanisms yet to be defined.

Our sequencing studies revealed recurrent mutations in a gene located in an MDS common deleted region on chromosome 5q. SNP array studies have not identified any genes on 5q that undergo homozygous deletion in del(5q) MDS (Gondek et al., 2008; Graubert et al., 2009; Heinrichs et al., 2009). Indeed, our studies would indicate that homozygous inactivation of *CSNK1A1* would be highly deleterious to a hematopoietic cell. Although *CSNK1A1* mutations in MDS are rare, they provide powerful evidence that these lesions are genetic drivers of clonal dominance. In functional studies, expression of the identified *CSNK1A1* E98V allele, in the setting of inactivation of both wild-type alleles to mimic the genetic context of the mutations observed in patients, caused an induction of nuclear β-catenin and a significant HSC cell cycle progression compared to expression of the wild-type *CSNK1A1*. Future experiments using a conditional knock-in mouse strain will be helpful to study the long-term hematopoietic effects of the mutant allele expressed at physiological levels.

Our results indicate that *CSNK1A1* is a CYCLOPS (copy number alteration yielding cancer liabilities owing to partial loss) gene (Nijhawan et al., 2012). Heterozygous inactivation of *Csnk1a1* sensitized cells to CK1 inhibition with D4476. The ablation of hematopoiesis in *Csnka1a1* null cells, and the normal to enhanced hematopoiesis in *Csnk1a1* haploinsufficient cells, provides a mechanistic basis for this therapeutic window. We demonstrated that systemic *Csnk1a1* haploinsufficiency in our murine model does not have significant effects on other organs, indicating that partial pharmacologic inhibition of CK1 would likely be well tolerated. While D4476 does not have pharmacokinetic properties for in vivo use, and lacks specificity for CK1α, a more selective compound has the potential for therapeutic utility in the treatment of patients with myeloid malignancies associated with del(5q).

Experimental Procedures

Generation of a *Csnk1a1* conditional knockout mouse

Mouse embryonic stem (ES) cells with *Csnk1a1* exon 3 targeted in a C57BL/6N genetic background were generated by the KOMP consortium (project ID: CSD45494). The neomycin/lacZ cassette was flipped out in vitro by transfection of a plasmid expressing the flippase recombinase (FRT). Successful FRT recombination was validated by PCR (forward primer: 5′-TCGCACTTGAGCTATTGGGGAGT-3′; reverse primer: 5′-AGGCATGGTAGCTCACACCTGA-3′). Following confirmation of germline transmission, mice were crossed with the *Mx1-Cre* mouse strain (Jackson: 002527). To excise *Csnk1a1* exon 3, *Csnk1a1* conditional mice were given three rounds of 200 μg of poly(I:C) (GE Healthcare Life Sciences) using intraperitoneal injections. Successful excision of *Csnk1a1* exon 3 was validated using forward primer above and reverse 5′ AGCTGGGCTACCAAGAGGCAA-3′ primer. All experiments and procedures were conducted in the Children's Hospital Boston animal facility and were approved by the Children's Hospital Institutional Animal Care and Use Committee.

Flow cytometry

Bone marrow (BM) cells were isolated by flushing and crushing pelvis and hind leg bones with PBS (GIBCO) + 2% FBS + Penicillin/Streptomycin (GIBCO). Whole bone marrow was lysed on ice with red blood cell (RBC) lysis solution (Invitrogen/Life Technologies), and washed in PBS (GIBCO) + 2% FBS. Single-cell suspensions of spleen were prepared by pressing tissue through a cell strainer followed by red blood cell lysis. Cells were labeled with monoclonal antibodies in 2% FBS/PBS for 30 min on ice (see Supplemental Experimental Procedures for the information on antibodies used) and analyzed using an LSRII (BD biosciences). Apoptosis (Annexin V apoptosis detection kit, ebioscience) and cell cycle (Ki67 cell cycle and proliferation kit, BD biosciences) assays were performed according to the manufacturer's instructions.

Bone marrow transplantation assays

In transplantation assays of *Csnk1a1* cells into CD45.1 wild-type mice, 5×10^6 freshly isolated whole bone marrow cells were harvested before poly(I:C) treatment, and injected into the tail-vein of lethally irradiated (1050 Rads) CD45.1-positive B6. SJL (Jackson) recipient mice without support cells. In competitive bone marrow transplantation studies, 2×10^6 freshly isolated bone marrow cells were harvested and transplanted via tail vein into lethally irradiated $CD45.1^+$ recipient mice together with 2×10^6 freshly isolated $CD45.1^+$ bone marrow competitor cells in an equal ratio. Four weeks after transplantation, blood samples were taken and donor cell chimerism was determined by flow cytometric analysis. Shortly thereafter, mice were given three rounds of poly(I:C) treatment and donor blood cell chimerism was determined every four weeks.

Western blots

Western blots were performed according to standard protocols. In brief, cell lysis was performed in RIPA buffer with protease/phosphatase inhibitors. After protein quantification, lysates were resuspended in Laemmli Sample Buffer, and loaded to gradient gels (Criterion Tris-HCl Gel, 8–16%). Proteins were transferred onto Immobilon polyvinyl difluoride (PVDF) membranes. As primary antibodies β-catenin (rabbit polyclonal, 9562, 1:500, Cell Signaling), p53 (mouse monoclonal, DO-1, 1:500, Santa Cruz), p21 (rabbit polyclonal, 1:200, C-19, Santa Cruz), Cyclin D1 (rabbit monoclonal, 1:200, SP4, Thermo Scientific) and GAPDH (rabbit polyclonal, 1:4000, Bethyl laboratories Inc) were applied.

Histopathology

For histological and immunohistochemical analyses, murine organs were fixed in 3.7% formaldehyde overnight, dehydrated and prepared for paraffin embedding. Hematoxylin-Eosin (H&E) staining was done according to routine protocols. For immunohistochemical stainings, the Avidin-Biotin Complex (ABC) was applied. Peripheral blood smears were stained with May-Grünwald-Giemsa (Sigma-Aldrich). Images were obtained on a Nikon Eclipse E400 microscope (Nikon, Tokyo, Japan) equipped with a SPOT RT color digital camera model 2.1.1 (Diagnostic Instruments).

Viral vector cloning

MIG-CSNK1A1, MIG-CSNK1A1(E98V) and MIG-CSNK1A1(D136N) were flanked by Not1 and Xho1 sites for convenient cloning into the MIG vector backbone.

Patient samples and Sequencing

Patients included in the whole exome sequencing were diagnosed between 2008 and 2013 at different Spanish hospitals affiliated to the MDS Spanish Group (*Grupo Español de SMD, GESMD*). Patients were diagnosed with MDS according to the French-American-British and 2008 World Health Organization classification. Samples were deidentified at the time of inclusion. This study was approved by institutional review boards (Clinical Research Ethics Committee Institut Català de la Salut/Germans Trias i Pujol Hospital and Clinical and Ethics Committee Parc de Salut MAR) and performed in accordance with the declaration of Helsinki. All patients gave their informed written consent. Whole-exome sequencing was performed using paired-end reads generated from DNA libraries prepared from MDS samples (whole bone marrow) with matched normal samples ($CD3^+$ lymphocytes isolated from peripheral blood). Whole-exome hybrid capture was carried out on 3 μg of genomic DNA, using the SureSelect Human Exome Kit version 3 (Agilent Technologies, Inc., Santa Clara, CA, USA). The captured exome library was sequenced with 100bp paired-end reads on an Illumina HiSeq2000 platform (Illumina, San Diego, CA, USA). Whole-exome sequencing data were analyzed using an in-house bioinformatics pipeline as previously reported (BWA; GATK's; VarScan2; SAMTools; SnpEff: (Koboldt et al., 2012; McKenna et al., 2010). Somatic mutations identified as alterations present in tumor but not in the matched $CD3^+$ sample were validated by Sanger sequencing. Sanger sequencing was performed on genomic DNA isolated from whole bone marrow cells and $CD3^+$ cells using GentraPuregene Cell kit (Qiagen, Valencia, CA, USA). Exon 3 from *CSNK1A1* gene was amplified by polymerase chain reaction (PCR) using the following primers: forward primer: 5′-TCCTTTTGTTTCGTTAGGTGGT-3′ and reverse primer 5′-AAGGTTAAATAGTGATGCACAGGA-3′; amplification size: 251 bp). Single nucleotide polymorphism array (SNP-A) were performed with Genome-Wide Human SNP Array 6.0 from Affymetrix. Assays were performed according to Affymetrix protocols.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- *Csnk1a1* haploinsufficiency increases HSC number and function
- *Csnk1a1* homozygous inactivation leads to bone marrow failure
- In del(5q) MDS, *CSNK1A1* is deleted, and *CSNK1A1* is a therapeutic target
- *CSNK1A1* is recurrently mutated in del(5q) MDS

Significance

Our studies provide functional and genetic evidence indicating that *CSNK1A1* plays a central role in the pathogenesis of del(5q) MDS. We found that heterozygous inactivation of *Csnk1a1* causes hematopoietic stem cell expansion and β-catenin activation. In addition, we found that *Csnk1a1* haploinsufficiency sensitizes cells to casein kinase inhibition, demonstrating an approach for the targeting of heterozygous deletions in cancer. While no recurrently mutated genes have been previously identified in genes within the common deleted regions of chromosome 5q, we found recurrent mutations in *CSNK1A1* in a subset of del(5q) MDS patients. In aggregate, these findings indicate that *CSNK1A*1 is a promising therapeutic treatment for the treatment of del(5q) MDS.

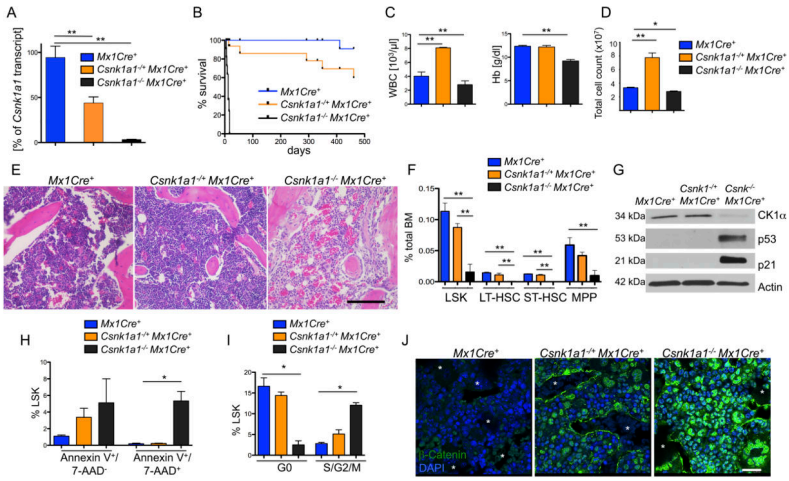

Figure 1. Conditional homozygous inactivation of *Csnk1a1* results in hematopoietic stem and progenitor cell ablation

(A) Deletion of *Csnk1a1* in whole bone marrow cells was determined 7 days after poly(I:C) induction by quantification of *Csnk1a1* transcript levels by qt-RT-PCR. Data is presented as remaining *Csnk1a1* transcript expression in percent relative to *Mx1Cre*$^{+}$-control mice (mean ±SD, n=3). (B) Kaplan-Meier survival curve of *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ (n=10), *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ (n=10) and *Mx1Cre*$^{+}$ (n=10) control mice. Time point 0 is the day of the first of 3 poly(I:C) inductions. (C) Absolute numbers of white blood cells (WBC) and hemoglobin (Hb) levels in peripheral blood from *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ controls 10 days after poly(I:C) induction (mean±SD, n=3, *p<0.05, **p<0.001). (D) Numbers of whole bone marrow cells collected from tibias, femurs and pelvis of *Csnk1a1*$^{-/+}$*MxCre*$^{+}$, *Csnk1a1*$^{-/-}$*MxCre*$^{+}$ 10 days after poly(I:C) induction (mean ±SD; n=3, *p<0.001). (E) Histological analysis of HE-stained spine from *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ controls 10 days after poly(I:C). Scale bar: 200 µm. (F) Analysis of the HSC compartment, defined as Lin^{low}Sca1^{+}ckit^{+} (LSK), long-term (LT; lin^{low}Sca1^{+}ckit^{+}CD48^{-}CD150^{+}), short-term (ST; lin^{low}Sca1^{+}ckit^{+}CD48^{-}CD150^{-}) HSC and multipotent progenitor cells (MPP, lin^{low}Sca1^{+}ckit^{+}CD48^{+}CD150^{-}) in the bone marrow from *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ controls 10 days after poly(I:C), (mean±SD, n=5, *p<0.05, **p<0.001). (G) Western blot of whole bone marrow lysate 8 days after induction of poly(I:C). (H) Apoptosis was assessed in the LSK fraction from BM by Annexin V and 7-AAD staining (early apoptosis: Annexin V^{+}/7-AAD^{-}; late apoptosis: Annexin V^{+}/7-AAD^{+}, (mean±SD, n=3, *p<0.05, **p<0.001). (I) Cell cycle was analyzed by combined proliferation (Ki67) and cell cycle (Hoechst 33342) staining in permeabilized LSK from bone marrow (G0: Ki67^{-}/Hoechst^{-}; S/G2/M: Ki67^{+}/Hoechst^{+}), (mean±SD, n=5, *p<0.05). (J) Immunofluorescence staining of paraffin-embedded bone marrow with an antibody against β-catenin (DAPI counterstaining). Asterisks highlight erythrocyte filled sinusoids. Scale bar: 20 µm. See also Figure S1.

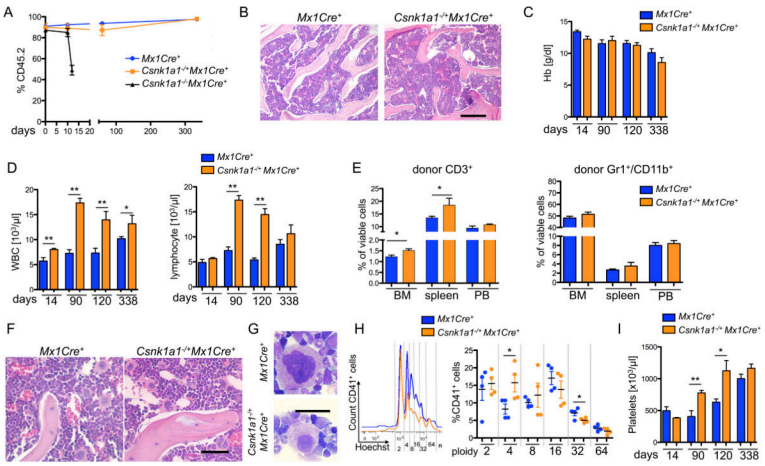

Figure 2. Homozygous *Csnk1a1* inactivation causes cell intrinsic hematopoietic stem cell ablation, while *Csnk1a1* heterozygous inactivation causes cell-intrinsic lineage expansion

(A) Donor chimerism (CD45.2) of *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ derived hematopoietic cells was monitored over time (mean±SD, n=7, **p<0.001). (B) Histomorphological analysis of transplanted *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ cells 8 weeks after poly(I:C) induction. Scale bar: 200 μm. (C) Hemoglobin (Hb) levels were followed over time (mean±SD, n=7, non-significant). (D) White blood cell and lymphocyte count were monitored over time (mean±SD, n=7, *p<0.05, **p<0.001). (E) Distribution of donor derived (CD45.2^{+}) myeloid (Gr1^{+}/CD11b^{+}) and T-cells (CD3^{+}) was analyzed by flow cytometry in bone marrow, spleen and peripheral blood (mean±SD, n=5, *p<0.05). (F) Histomorphological analysis of megakaryocyte dysplasia in transplanted *Mx1Cre*$^{+}$ and *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ 8 weeks after poly(I:C) induction. Scale bar: 100 μm. (G) Detailed megakaryocyte morphology on cytospin preparations (May-Gruenwald-Giemsa staining, Oil immersion, Scale bar: 20 μm). (H) Representative ploidy analysis and quantification on CD45.2^{+}, CD41^{+} megakaryocytes using Hoechst33342 staining on fixed and permeabilized cells. (mean±SD, n=4, *p<0.05). (I) Platelet counts were taken over time (mean±SD, n=7, *p<0.05). See also Figure S2.

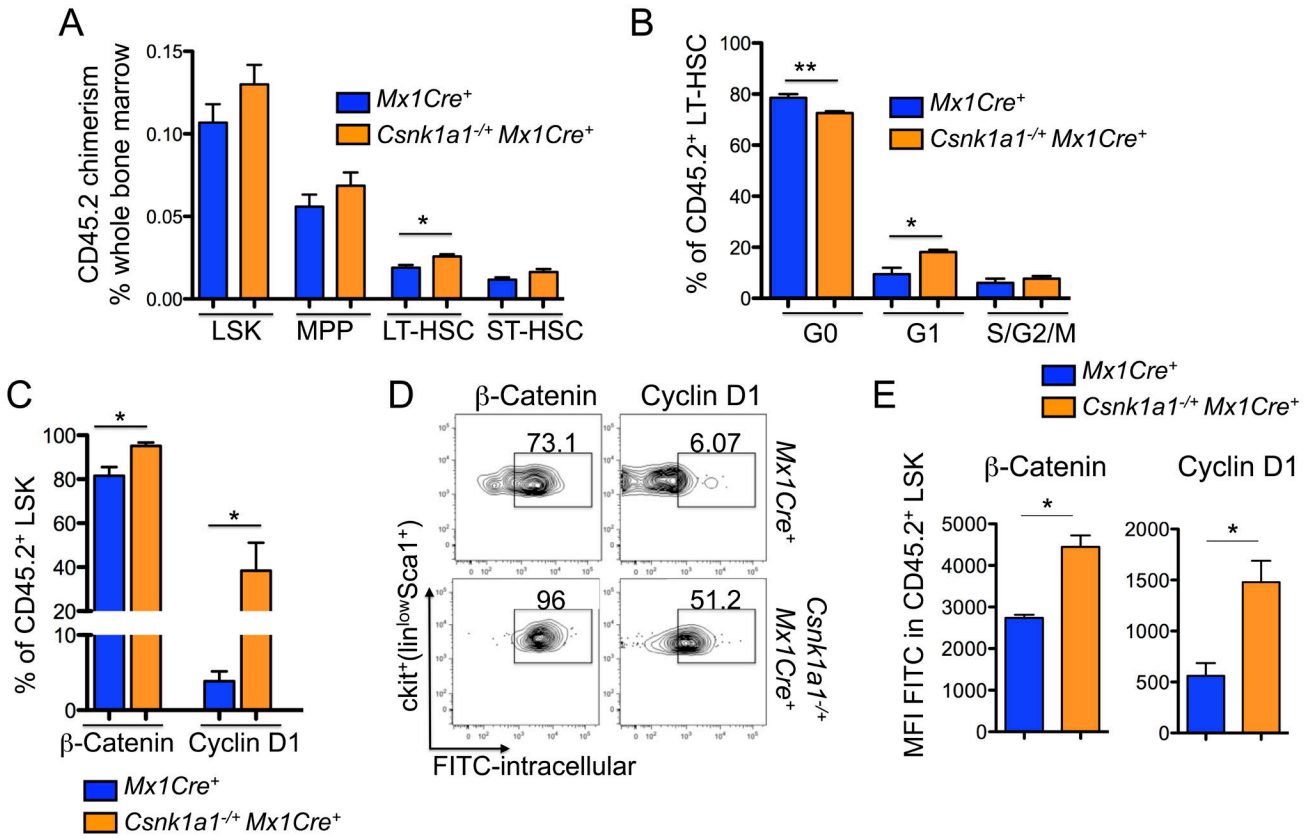

Figure 3. Haploinsufficiency of *Csnk1a1* leads to cell-intrinsic expansion of transplanted hematopoietic stem cells

(A) HSC chimerism (CD45.2) was analyzed in CD45.1 mice repopulated with *Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$ and *Mx1Cre*$^{+}$ cells 8 weeks after induction with poly(I:C) in the LSK, MPP, LT-HSC, and ST-HSC (mean±SD, n=5, *p<0.05). (B) Cell cycle was analyzed by combined proliferation (Ki67) and cell cycle (Hoechst 33342) staining in permeabilized LSK and LT-HSC from bone marrow (G0: Ki67^{-}/Hoechst^{-}; S/G2/M: Ki67^{+}/Hoechst^{+}), (mean±SD, n=5, *p<0.05). (C) Intracellular flow cytometry for β-catenin and cyclin D1 (FITC-labeled secondary antibody each) on the CD45.2^{+} viable LSK population (mean±SD, n=3, *p<0.05). (D) Corresponding representative flow blots to the quantitative analysis of intracellular β-catenin and cyclin D1, accumulation. (E) Mean fluorescence intensity (MFI) of intracellular β-catenin and cyclin D1 in LSK (mean±SD, n=3, *p<0.05). See also Figure S3.

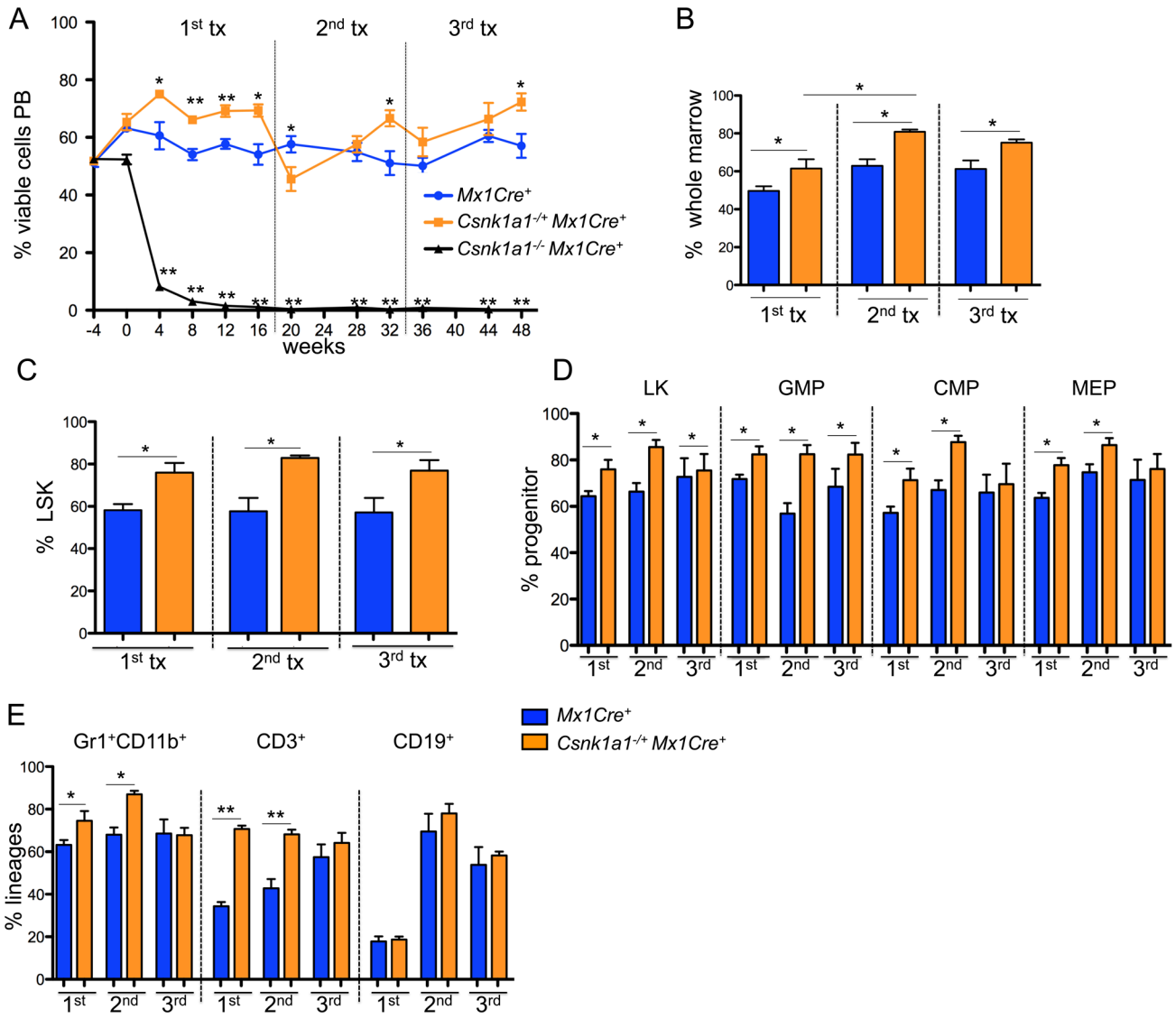

Figure 4. *Csnk1a1* haploinsufficient hematopoietic stem cells show increased repopulating ability consistent with increased self-renewal

(A) Competitive repopulation assays were performed by mixing CD45.2-expressing cells (*Csnk1a1*$^{-/+}$*Mx1Cre*$^{+}$, *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$, or *Mx1Cre*$^{+}$) with CD45.1 competitor cells at an approximately 50:50 ratio, and transplanting the cells into lethally irradiated CD45.1 recipients. The percentage of CD45.2 donor cell chimerism in the whole peripheral blood from peripheral blood of lethally irradiated recipient animals is shown. Time (weeks) denotes the time relative to termination of the poly(I:C) injections (poly(I:C)=timepoint 0). After 16 weeks, bone marrow was harvested and transplanted for secondary transplants, and 16 weeks later for tertiary transplants in lethally irradiated mice (mean±SD, n=5, *p<0.05; **p<0.001). (B) Donor chimerism of total bone marrow cells performed at 16 (first competitive transplant), 32 (secondary competitive transplant, 16 weeks after transplantation) or 48 (tertiary competitive transplant) weeks after poly(I:C) induction (mean ±SD, n=5, *p<0.05). (C, D) Donor chimerism of the hematopoietic stem (LSK) (C) and

progenitor cell compartments: LK, $lin^{low}Sca1^{-}ckit^{+}$; common-myeloid progenitors (CMP), LK $CD34^{+}CD16/32^{-}$; granulocyte-macrophage progenitors (GMP), LK $CD34^{+}CD16/32^{+}$; myeloerythroid progenitors (MEP), LK $CD34^{-}CD16/32^{-}$ (D) performed at 16 (first competitive transplant), 32 (secondary competitive transplant), or 48 (tertiary competitive transplant) weeks after poly(I:C) induction (mean±SD, n=5, *p<0.05). (E) Chimerism of hematopoietic lineages in the bone marrow each 16 weeks after the first, second and third competitive transplant. Composite data of donor ($CD45.2^{+}$) granulocytes ($Gr1^{+}CD11b^{+}$), B-cells ($CD19^{+}$) or T-cells ($CD3^{+}$) are shown (mean±SD, n=5, *p<0.05).

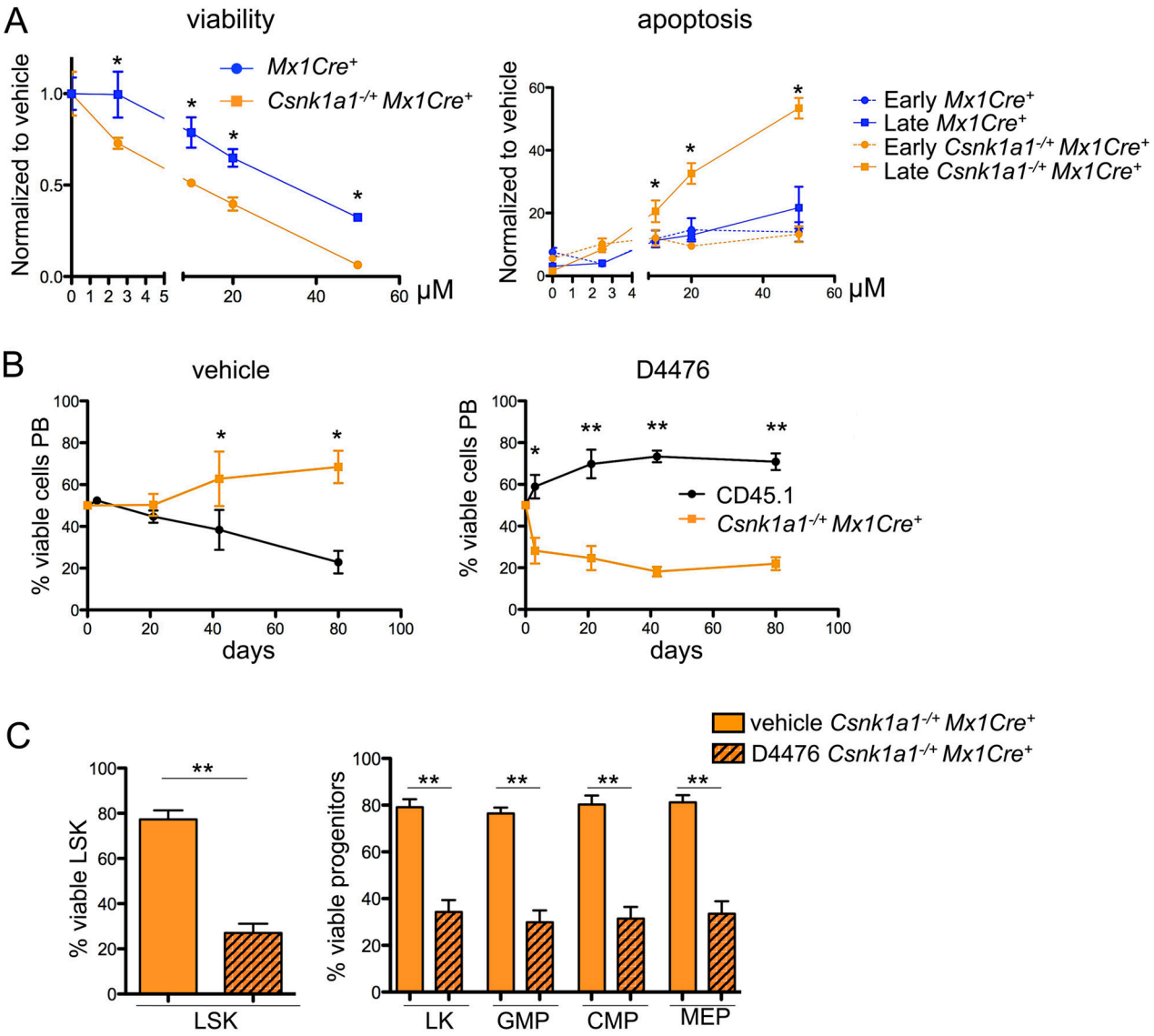

Figure 5. *Csnk1a1* haploinsufficiency provides a therapeutical window for the specific treatment of disease-propagating hematopoietic stem cells

(A) Sorted hematopoietic progenitor cells (LK) were pre-stimulated for 24 hours after the sort and treated for 72 hours with varying concentrations of D4476. Viability of cells was analyzed after 72 hours with the CellTitre glo assay, apoptosis by combined Annexin V and 7AAD staining discriminating early (Annexin V^+7AAD^-) and late apoptosis ($AnnexinV^+7AAD^+$) using flow cytometry. (mean±SD, n=5, *p<0.05). (B) 21 days after poly(I:C) treatment, *Csnk1a1*$^{-/+}$*Mx1Cre*$^+$ or CD45.1 bone marrow was harvested and LSK cells were sort-purified. Equal ratios of *Csnk1a1*$^{-/+}$*Mx1Cre*$^+$ and $CD45.1^+$ LSK were mixed and treated for 72 hours ex vivo with either D4476 or DMSO, followed by transplantation into lethally irradiated CD45.1 mice. The chimerism was followed over time in the peripheral blood (mean±SD, n=6, *p<0.05; **p<0.001). (C) The chimerism of *Csnk1a1*$^{-/+}$*Mx1Cre*$^+$ in the bone marrow was analyzed in the LSK and progenitor fractions

after 12 weeks under DMSO or D4476 treatment conditions (mean±SD, n=6, **p<0.001). See also Figure S4.

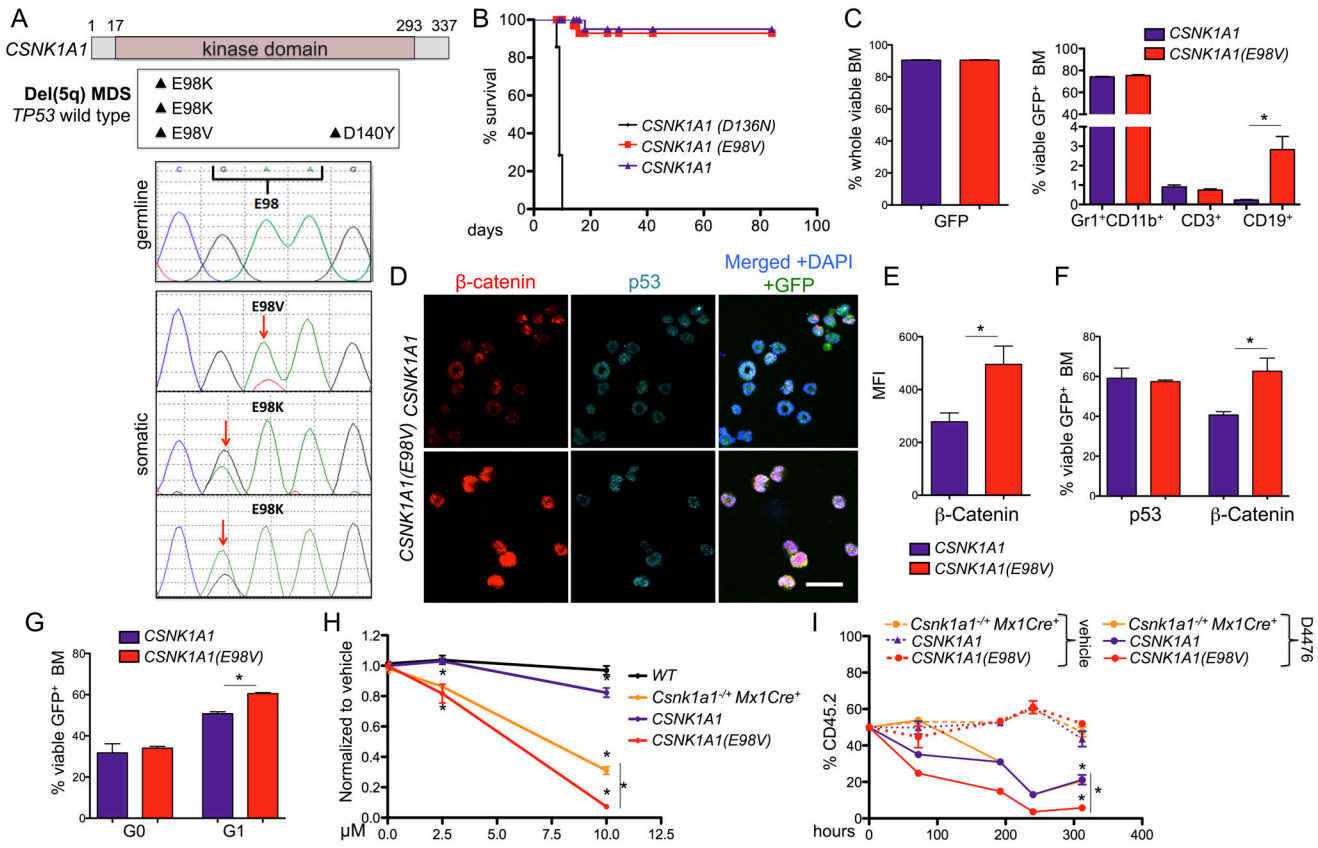

Figure 6. Identification and functional characterization of *CSNK1A1* mutations in del(5q) MDS patients

(A) Summary of *CSNK1A1* mutations identified in del(5q)MDS patients (upper panel) and Sanger sequencing results around codon 98 of the normal control (germline) and tumors (somatic) (lower panel). (B) Kaplan-Meier survival analysis of chimeric mice transplanted with *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ hematopoietic stem and progenitor cells expressing *Csnk1a1* cDNA, *Csnk1a1* D136 cDNA or *Csnk1a1* E98V cDNA. Timepoint 0 is the first day of poly(I:C) induction (n=5). (C) GFP expression in whole BM (left) and distribution of the different lineages (Gr1^{+}CD11b^{+} neutrophils, CD3^{+} T-cells, CD19^{+} B-cells) in GFP^{+} BM cells (right). (mean±SD, n=3, *p<0.05). (D) Co-immunofluorescent staining of β-catenin and p53 in cytospin preparations of red blood cell lysed whole bone marrow cells (red: β-catenin, turquoise: p53, green: GFP MIG vector, blue: DAPI). Scale bar 20 μm. (E) Quantification of β-catenin intensity using the mean fluorescence intensity (MFI), (mean ±SD, n=3, *p<0.05). (F) Intracellular flow cytometry measurement of β-catenin and p53 in permeabilized whole bone marrow cells. (mean±SD, n=3, *p<0.05). (G) Cell cycle was analyzed by combined Ki67 and Hoechst33342 staining in permeabilized whole bone marrow cells. (mean±SD, n=3, *p<0.05). (H) GFP^{+} LSK from mice transplanted with either *CSNK1A1* or *CSNK1A1* E98V-expressing *Csnk1a1*$^{-/-}$*Mx1Cre*$^{+}$ cells as well as *Csnk1a1* haploinsufficient LSK and WT LSK were sort purified and exposed to vehicle, 2.5 or 10 μM D4476 for 72 hours and viability of cells was analyzed after 72 hours with the CellTitre glo assay (mean±SD, n=5, *p<0.05). (I) LSK (all CD45.2) isolated as in (H) were treated in competition to CD45.1 wild type cells in one culture well to analyze selective ablation of

cells under the same culture condition. (mean±SD, n=5, *p<0.05). See also Figure S5 and Table S1.

Table 1

Clinical data of patients with the identified somatic *CSNK1A1* mutations

	#1	#2	#3
Age at diagnosis	85	82	65
Gender	M	F	F
FAB	RA	RA	RA
Karyotype	46, XY, del(5)(q13q31)[17]/46, XY[3]	46, XX, del(5)(q13q33)[14]/46, XX[6]	46, XX, del(5)(q13q33)[4]/46, XX[2]
IPSS-R	2, low risk	1, very low risk	1, very low risk
TP53	wild type	wild type	wild type
Hemoglobin [g/dL]	10.6	11.8	9.5
Absolute neutrophil count [10^9/l]	49.6	0.951	5.01
Platelets [cells/μl]	140,000	118,000	72,000

M: Male; F: Female; FAB: French-American-British classification; RA: Refractory Anemia. IPSS-R: revised International Prognostic Scoring System