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Bone progenitor dysfunction induces myelodysplasia and secondary leukemia

Marc H.G.P. Raaijmakers^{1,2,3,*}, Siddhartha Mukherjee^{1,2,3,4,*#}, Shangqin Guo^{1,2,3}, Siyi Zhang^{1,2,3}, Tatsuya Kobayashi⁵, Jesse A. Schoonmaker^{1,2,3}, Benjamin L. Ebert⁶, Fatima Al-Shahrour⁶, Robert P. Hasserjian⁷, Edward O. Scadden^{1,2,3}, Zinmar Aung^{1,2,3}, Marc Matza^{1,2,3}, Matthias Merckenschlager⁸, Charles Lin⁹, Johanna M. Rommens¹⁰, and David T. Scadden^{1,2,3,4}

¹Center for Regenerative Medicine, Massachusetts General Hospital and Harvard Medical School CPZN - Room 4265A, 185 Cambridge Street, Boston, MA 02114

²Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138

³Harvard Stem Cell Institute, Cambridge MA 02138

⁴Cancer Center, Massachusetts General Hospital, Boston MA 02114

⁵Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

⁶Hematology Division, Brigham and Women's Hospital, Boston MA 02115 and Broad Institute, Cambridge, MA 02138

⁷Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

⁸Lymphocyte Development Group, Medical Research Council Clinical Sciences Centre, Imperial College London, Du Cane Road, London W12 0NN, United Kingdom

⁹Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

¹⁰Program in Genetics and Genome Biology, The Hospital for Sick Children, Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

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Address correspondence to: Marc H.G.P. Raaijmakers, 185 Cambridge Street, Boston, Massachusetts 02114, USA, hraaijmakers@partners.org or David T. Scadden, 185 Cambridge Street, Boston, Massachusetts 02114, USA, dscadden@mgh.harvard.edu.

*These authors contributed equally to this work.

#Current address: Department of Medicine and Irving Cancer Research Center, Columbia University School of Medicine, New York 10032

Author contributions

M.R., S.G. and D.S. initiated the study. M.R., S.M. and D.S. designed the experiments and analyzed the data. M.R. carried out most of the experimental work with the help of S.M., J.S., T.K., S.G., E.S., S.Z., M.M., Z.A., and J.F. B.E. and F.A. analyzed the microarray results. R.H. reviewed bone marrow histology and peripheral blood morphology. C.L. supervised the in vivo imaging studies. M.M. and J.R. provided materials and discussion, M.R. and D.S. wrote the manuscript. D.S. directed the research. All authors discussed and commented on the manuscript.

Abstract

Mesenchymal cell populations contribute to microenvironments regulating stem cells and the growth of malignant cells. Osteolineage cells participate in the hematopoietic stem cell niche. Here, we report that deletion of the miRNA processing endonuclease *Dicer1* selectively in mesenchymal osteoprogenitors induces markedly disordered hematopoiesis. Hematopoietic changes affected multiple lineages recapitulating key features of human myelodysplastic syndrome (MDS) including the development of acute myelogenous leukemia. These changes were microenvironment dependent and induced by specific cells in the osteolineage. *Dicer1*^{-/-} osteoprogenitors expressed reduced levels of *Sbds*, the gene mutated in the human bone marrow failure and leukemia predisposition Shwachman-Bodian-Diamond Syndrome. Deletion of *Sbds* in osteoprogenitors largely phenocopied *Dicer1* deletion. These data demonstrate that differentiation stage-specific perturbations in osteolineage cells can induce complex hematological disorders and indicate the central role individual cellular elements of ‘stroma’ can play in tissue homeostasis. They reveal that primary changes in the hematopoietic microenvironment can initiate secondary neoplastic disease.

Mesenchymal cells are a part of virtually every tissue in metazoans and are thought to participate in organ formation, cellular composition, patterning and size. In adult tissues, these cells are considered ‘stroma’ without clear function. Identification of somatic stem cell populations has made possible the study of heterologous cells comprising their niche. Mesenchymal cells of osteolineage have been shown to regulate hematopoietic stem cells (HSC) ^{1,2} with *osterix* expressing skeletal progenitors initiating ectopic HSC niche formation³. We previously examined mesenchymal-HSC interactions using mice engineered to express individual genes in osteoblastic cells^{1,4} and now sought to define more precisely the stage of osteolineage differentiation critical for hematopoiesis using promoters more restricted in their expression to alter candidate genes. We chose to alter a gene that could modulate a landscape of other gene products.

Dicer1 is a RNase III endonuclease essential for miRNA biogenesis⁵ and RNA processing⁶. MiRNAs regulate hematopoietic cell fate⁷ and global down-regulation of miRNAs by *Dicer1* deletion promotes tumorigenesis in a cancer-cell-autonomous manner⁸. We used *Dicer1* deletion as a means of altering multiple gene products in subsets of mesenchymal osteolineage cells. We intercrossed mice expressing GFP-*Cre* recombinase under the transcriptional control of the *osterix*⁹ promoter expressed in osteoprogenitor cells to mice with floxed *Dicer1* alleles¹⁰ generating Osx-GFP-Cre⁺; *Dicer*^{fl/+} (OCD^{fl/+} control) and Osx-GFP-Cre⁺ *Dicer*^{fl/fl} (OCD^{fl/fl} mutant) mice. The Cre transgene in this model is expressed coordinately with endogenous Osterix¹¹ (figure 1a). Mutant mice were born at Mendelian frequency, but displayed growth retardation and impaired survival (~30% mutant mortality by 8 weeks). Therefore, we examined mice at age 4–6 weeks.

Impaired osteoblastic differentiation

Dicer1 gene deletion in bone was shown by genomic PCR (figure 1b) and its efficiency confirmed by qPCR of *Dicer1* mRNA in primary GFP+ (CD45⁻lineage⁻CD31⁻) cells (figure 1c,d and supplementary figure 1a,b). *Dicer1* was not deleted in hematopoietic stem

and progenitor cells (figure 1e) nor expressed in other hematopoietic subpopulations (not shown). Bone marrow stromal osteogenic colony number was decreased relative to total colony forming number (CFU-ALK/CFU-F), indicative of reduced osteogenic differentiation (figure 1f,g). $OCD^{fl/fl}$ bone marrow stromal cultures also demonstrated decreased alkaline phosphatase and calcified matrix deposition upon osteogenic differentiation (supplementary figure 1d). *In vivo*, osteoblasts from $OCD^{fl/fl}$ mice expressed less of the terminal differentiation marker *osteocalcin* (figure 1h and supplementary figure 1e). Altered texture of the mineralized bone matrix was observed (supplementary figure 1f), but not reduction in trabecular or cortical bone volume (supplementary figure 1g,h). Osteoblast number was modestly, although significantly decreased, whereas osteoclast number and function was not affected (supplementary figure 1i–k and not shown). Taken together, the data show that *Dicer1* deletion in osteoprogenitors impairs osteoblastic differentiation both *in vitro* and *in vivo*.

Myelodysplasia in $OCD^{fl/fl}$ mice

Leukopenia with reduced numbers of cells in all leukocyte subsets was found in $OCD^{fl/fl}$ mice (figure 2a and supplementary figure 2a). Erythrocyte and platelet counts varied widely in $OCD^{fl/fl}$ mice with some animals showing profound anemia and thrombocytopenia (figure 2a). The variability was at least partly explained by extramedullary hematopoiesis in the spleen (supplementary figure 3) mitigating cytopenia in those animals. The bone marrow of $OCD^{fl/fl}$ mice was normo- to hypercellular (supplementary figure 2c,d). No significant differences were found in the frequency of immunophenotypically defined hematopoietic stem ($Lineage^{-}ckit^{+}Sca1^{+}(LKS)CD150^{+}CD48^{-}$) and progenitor (LKS) cells (supplementary figures 2d,e and 4). Limiting dilution transplant analysis revealed an increase in HSCs in mutants which did not reach statistical significance (1:29000 vs. 1:52000, $p=0.14$). HSC and progenitor function was not impaired in $OCD^{fl/fl}$ mice as demonstrated by competitive reconstitution capacity upon transplantation or colony-forming unit (CFU-C) analysis (supplementary figure 2f,g). Decreased blood cell counts despite normal bone marrow cellularity and normal to increased stem cell function is characteristic of ineffective hematopoiesis.

Morphologic assessment revealed marked dysplasia with nuclear hypersegmentation in neutrophils and giant platelets in the blood and micromegakaryocytes with hypolobulated, hyperchromatic nuclei in the bone marrow of $OCD^{fl/fl}$ mice (figure 2b,c and supplementary figure 5a–c). There was neither increased fibrosis on reticulin staining nor ring sideroblasts on iron stains of the bone marrow (not shown). Peripheral cytopenia with dysgranulopoiesis and dysplastic megakaryocytes is consistent with a diagnosis of MDS in mice according to the Bethesda criteria¹². The aetiology of human MDS is complex and poorly understood. It has been very difficult to engraft and propagate the hematopoietic characteristics of human MDS in murine models by transplanting hematopoietic cells from MDS patients into immunodeficient mice, sparking a longstanding debate about a potential causative or facilitating role of the microenvironment in the pathogenesis of this disease.

Examining other key characteristics of the human disease, we observed increased apoptosis and proliferation of primitive hematopoietic progenitors (figure 2d,e and supplementary

figures 6, 7 and 8a)¹³. Increased apoptosis was limited to primitive progenitor cells, as no differences were observed in differentiated (lineage +) cells (not shown). Within Lineage-ckit+ cells, apoptosis was most pronounced in megakaryocyte-erythroid progenitors (MEP) (supplementary figure 7). HSC generally are more quiescent in close proximity to the endosteal surface *in vivo*.¹⁴ When we imaged transplanted wild-type cells, we noted that LKS cells located significantly closer to the endosteum in mutant animals compared with controls (supplementary figure 8b,c) and the frequency of cell duplets, an indicator of cell proliferation, was strikingly increased (supplementary figure 8d), demonstrating increased proliferation of primitive progenitors despite close proximity to the endosteal surface.

OCD^{fl/fl} mice also had reduced B-cells and B-cell progenitors with a concomitant increased frequency of myeloid cells in the bone marrow (supplementary figures 2h,i and 9) as observed in human MDS^{15,16}. The nature of the B-cell defect in early MDS has remained elusive since cytogenetic studies have failed to link B-cells to the malignant clone in MDS. Interestingly, osteoblasts have recently been shown to constitute a niche for B-cell lymphopoiesis^{17,18}. OCD^{fl/fl} mice also displayed variable, but increased vascularity in the bone marrow, a feature that has been described in MDS¹⁹ (supplementary figure 2j). Deficiency of either vitamin B12 or folate which can cause myelodysplastic features¹³, were excluded as contributing factors in the OCD^{fl/fl} mice (not shown).

Myelodysplasia is environmentally induced

Next, we used transplantation to assess the contribution of the microenvironment to the hematopoietic phenotype. Hematopoietic cells of OCD^{fl/fl} (CD45.2) mice demonstrating profound cytopenia and myelodysplastic features were transplanted into (CD45.1) B6.SJL mice (“wild-type environment”) (figure 3a). Transplanted animals were without apparent signs of disease. Assessment of peripheral blood and bone marrow 16 weeks (figure 3a–d and supplementary figure 10) or 12 months (supplementary figure 11) after transplant showed nearly complete donor (CD45.2) chimerism with complete normalization of cytopenias, granulocyte and megakaryocyte morphology, intramedullary apoptosis of primitive progenitors and bone marrow vascularity. The results indicate that the hematopoietic phenotype cannot be propagated in a hematopoietic cell-autonomous manner. Conversely, when bone marrow cells from wild-type B6.SJL mice were transplanted into lethally irradiated mutant or control mice (figure 3e–h and supplementary figure 12), mutant recipients developed leukopenia, anemia and thrombocytopenia, dysplastic features of CD45.1 blood cells, increased bone marrow vascularity with dysplastic megakaryocytes, increased frequency of myeloid cells, decreased frequency of B-cell subsets and increased apoptosis of primitive hematopoietic progenitors. The combined experiments provide formal proof that the myelodysplastic phenotype observed in mutant mice is determined by the micro-environment.

Osteolineage stage specificity

To examine whether mesenchymal cells of the osteoblastic lineage can directly induce aberrant hematopoiesis, we established bone marrow derived stromal cell cultures from OCD^{fl/+} vs. OCD^{fl/fl} mice and co-cultured these with red-fluorescent (DS-red)

hematopoietic LKS and MEP cells (supplementary figure 13). Stromal cells were osteolineage committed as evidenced by *Osterix* expression (and deletion of *Dicer1*; not shown) but lacked terminally differentiated osteoblasts as indicated by the absence of *osteocalcin* expression (supplementary figure 13a). Co-cultures on osterix⁺ osteocalcin⁻OCD^{fl/fl} stromal cells revealed increased proliferation of LKS cells (supplementary figure 13 b–d) and impaired megakaryocyte differentiation of MEP cells (supplementary figure 13 e–h). The latter was accompanied by morphological changes in megakaryocytic cells (supplementary figure 13i), resembling those observed *in vivo*. Together, the data show that the disruption of hematopoiesis observed in OCD^{fl/fl} mice *in vivo* is, at least partly, the result of a direct effect of osterix⁺osteocalcin⁻ osteolineage cells on hematopoietic cell proliferation and differentiation. To further explore whether osteoprogenitor cells rather than terminally differentiated (osteocalcin⁺) osteoblasts were the key participants in the hematopoietic abnormality, we crossed *Dicer1*^{fl/fl} with mice in which *cre* recombinase was driven by the *osteocalcin* promoter. Efficient recombination by the osteocalcin-*cre* as previously demonstrated²⁰ was confirmed (supplementary figure 14a) and serial dilution of DNA isolated from long bones demonstrated equivalent efficacy of *Dicer1* deletion using either the osteocalcin or osterix driven *cre* (not shown). *Dicer1* deletion in this genetic context recapitulated some aspects of the bone phenotype, notably the altered texture and increased volume of cortical bone (supplementary figure 14b,c), but did not result in any hematopoietic abnormalities (supplementary figure 14d–h). The data demonstrate that osteoprogenitor cell-specific effects of *Dicer1* deletion underlie the disruption of hematopoiesis and indicate that select subsets of cells in the osteoblast lineage have discrete functions in regulating hematopoiesis.

Emergence of leukemia

Human MDS is characterized by transformation to acute myeloid leukemia in a significant subset of patients. Strikingly, we observed the infrequent occurrence of myeloblastic tumors in OCD^{fl/fl} mice. Four mice were observed to have facial tumors (estimated frequency 2/100 OCD^{fl/fl} animals) of which three animals were available for detailed analysis (figure 4a). Tumors appeared at the age of 2–3 weeks and mice died at the age of 4–5 weeks. Analyses in a separate cohort of animals demonstrated that dysplastic features were already present in three week old animals (supplementary figure 15), suggesting that tumorigenesis occurred in the context of existing dysplasia. Histological examination of the tumors established a diagnosis of myeloid sarcoma (figure 4b), a soft-tissue complication of acute myelogenous leukemia (AML). *Dicer1* had not been deleted in these myeloid sarcomas as determined by genomic PCR (figure 4c). Comparative genomic hybridization to germline DNA revealed genetic abnormalities in all tumors, providing direct evidence that the micro-environment facilitated clonal evolution of tumor cells (figure 4d and supplementary figure 16). Cytogenetic abnormalities included amplification of a common (27 Kb) region on chromosome 14qC1 in two tumors, the biological relevance of which, in the absence of known genes related to tumorigenesis in this region, is under investigation.

The myeloid sarcomas displayed infiltrative behavior into surrounding tissues (supplementary figure 17) and were diagnosed in a context of myelocytosis (figure 4e), anemia (figure 4f), profound splenomegaly with infiltration of blasts (figure 4g,h) and a

marked increase in blasts with a monocytic appearance in blood and bone marrow (figure 4i–k). This combination of findings strongly resembles monoblastic AML (FAB subtype AML M4/M5) in humans and fulfilled all criteria for the diagnosis of AML in mice¹². Although the presence of myeloid sarcoma and acute monocytic leukemia-like disease in $OCD^{fl/fl}$ mice was sporadic, myeloid sarcomas were never observed in $OCD^{fl/+}$ or $Osx-Cre$ -mice (which were four-fold more abundant than mutant animals) and the occurrence of spontaneous AML in mice at an age of 2–3 weeks is unprecedented to our knowledge. Taken together, these data demonstrate that disruption of *Dicer1* from cells of the osteoblastic lineage causes myelodysplasia in mice recapitulating key characteristics of human MDS, including the propensity to develop AML.

Deletion of Shwachman-Diamond-Bodian Syndrome gene

To obtain insight into the genes and molecular pathways in osteolineage cells driving the disruption of hematopoiesis in $OCD^{fl/fl}$ mice, we performed gene expression profiling of primary $OCD^{fl/+}$ and $OCD^{fl/fl}$ osteolineage cells ($GFP^+CD45^-lin^-CD31^-$). Microarray analysis revealed a broad changes in the transcriptome in $OCD^{fl/fl}$ osteolineage cells with differential expression of 656 genes (p-value = 0.05, Student's t test) representing a wide array of functional groups (supplementary table 1). Gene set enrichment analysis (GSEA) revealed significant enrichment (FDR < 0.05) of genes involved in early osteoblastic differentiation and commitment²¹, including the Wnt/ β catenin⁹ and TGF- β signaling pathway²², in $OCD^{fl/+}$ samples (supplementary table 2). Notably differentially expressed genes and pathways further included stress response genes and cytokines (supplementary tables 2 and 3) with significant downregulation of the Shwachman-Diamond-Bodian Syndrome gene (*Sbds*) (2.43-fold; p=0.028, table S1) of particular interest because of its association with human disease. Inactivating mutations in the Shwachman-Bodian-Diamond Syndrome (*Sbds*) gene are highly associated with the clinical syndrome²³ characterized by skeletal abnormalities, bone marrow failure with mild myelodysplastic changes, the propensity to develop MDS and secondary AML, increased intramedullary apoptosis of hematopoietic progenitors and exocrine pancreatic dysfunction. Studies knocking down the *Sbds* gene in hematopoietic progenitor subsets have failed to recapitulate key features of the clinical phenotype²⁴ and a role of the bone marrow micro-environment in the pathogenesis of the syndrome has been suggested²⁵. Targeted deletion of *Sbds* from osteoprogenitor cells by intercrossing Osterix-Cre mice to mice containing conditional (floxed) *Sbds* alleles (figure 5a) (S. Zhang *et al*, manuscript submitted) resulted in altered texture of the cortical bone (supplemental figure 18a), leukopenia and lymphopenia (but not neutropenia) (figure 5b), dysplastic features of neutrophils in the peripheral blood (figure 5c) and micromegakaryocytes (figure 5d) with increased vascularity of the bone marrow (figure 5e). Further similarities with $OCD^{fl/fl}$ mice included decreased frequency of B-cells and B-cell progenitors (supplementary figure 18b), increased frequency of myeloid cells (supplementary figure 18c) and intramedullary apoptosis of hematopoietic progenitor cells (figure 5f). Collectively, the data show that conditional deletion of *Sbds* from osteoprogenitor cells recapitulates key characteristics of $OCD^{fl/fl}$ mice and implicate osteoprogenitor cells in the pathogenesis of the Shwachman-Diamond syndrome.

DISCUSSION

These studies show that specific changes in specific mesenchymal cells of the hematopoietic microenvironment may be sufficient to initiate a complex phenotype of disordered homeostasis with similarities to myelodysplasia, a poorly understood human disease. Further, we demonstrate the ability of this abnormality to result in the emergence of a clonal neoplasm in a cell type of clearly distinct lineage with distinct secondary genetic changes. The data indicate that individual, well defined, mesenchymal microenvironment constituents can be primary enablers of neoplastic changes in a heterologous cell type.

While a series of genetic and epigenetic events in a single cell may be necessary for oncogenesis, they may not be sufficient and a permissive microenvironment has been hypothesized to be required for frank malignancy to emerge²⁶. Examples of microenvironmental contributions to neoplasia include a necessary mast cell contribution to Nf1 induced neurofibromas²⁷ and mesenchymal cell alteration of epithelial tumor growth kinetics^{28,29}. Changes in a tissue microenvironment have also been suggested to precede and promote the initiation of genetic events by creating a “pre-malignant” state characterized by disruption of quiescence inducing signals or increases in proliferative signaling^{30,31}. This has been validated experimentally with altered TGF- β signaling in tissue fibroblasts³² and with myeloid progenitor expansion following RAR- γ deletion in bone marrow or Rb deficiency in hematopoietic and microenvironmental cells^{33,34}.

Our findings further the paradigm of malignancy resulting from the interplay of cell autonomous and microenvironmentally determined events and point to the microenvironment as the site of the initiating event that leads to secondary genetic changes in other cells. It is therefore possible to envision a ‘niche-based’ model of oncogenesis whereby a change in a specific microenvironmental cell can serve as the primary moment in a multi-step process toward malignancy of a supported, but distinct cell type. Signals from the microenvironment may select for subsequent transforming events and therefore such signals may represent candidate therapeutic targets in both treatment and prevention strategies.

Whether osteoprogenitor cell abnormalities play a role in the pathogenesis of human MDS and leukemia cannot be discerned from our studies. MDS is a heterogeneous disorder in which cytogenetics can be normal or reveal specific clonal lesions such as deletion of 5q. A role for the microenvironment in MDS has been raised, and reduced osteoblast surface and unchanged bone volume similar to our OCD^{fl/fl} model has been reported³⁵. Our findings raise the possibility that microenvironmental alterations may precede and facilitate clonal evolution in MDS. Studies examining patient samples will explore this possibility.

The *Sbds* studies provide further suggestive links to human disease. The human syndrome combines features seen in our model and raises the issue of whether both the pathophysiology of the disorder and the difficulty in treating this syndrome with bone marrow transplantation is in part due to a microenvironmental defect in the bone marrow.

The function of *Sbds* is itself unclear, but it has been implicated in ribosomal biogenesis²³ and may therefore share with *Dicer1* the ability to modulate the gene expression

'landscape.' While we pursued *Sbds* because its expression was decreased in the absence of *Dicer1*, levels in *OCS^{fl/fl}* are likely to be even lower and as a consequence, may not faithfully portray the role of *Sbds* in our model. We therefore regard *Sbds* as a candidate participant in the phenotype of *OCD^{fl/fl}* mice, but further studies will be needed to define the collaborating molecules causing the hematopoietic abnormalities.

These studies were initiated focusing on the stem cell niche, but the results indicate that individual microenvironment constituents can serve as regulators of tissue functions beyond that of stem cell support. Osteoprogenitor dysfunction induced altered proliferation and differentiation of both HSCs and distinct hematopoietic progenitor subsets and induced changes in the tissue architecture. Within a tissue microenvironment, certain elements may have far broader, integrative functions as the osteoprogenitor cells did here. There may be a hierarchy of activity within niche components as well as the cells they regulate. Our studies provide the rationale for further exploration of the complexity of mesenchymal 'stroma' and the role specific mesenchymal subsets may play as primary regulators of normal and disordered tissue function.

METHODS SUMMARY

Mice

Osx-Cre transgenic mice⁹, *Ocn-Cre* transgenic mice²⁰ and floxed *Dicer1* mice¹⁰, have been described. B6.SJL-*Ptprc^a Pep3^b*/BoyJ mice were purchased from the Jackson Laboratory.

Transplantation

For competitive transplantation, 5×10^5 bone-marrow cells from 6-week-old *OCD^{fl/+}* or *OCD^{fl/fl}* (CD45.2) littermates admixed with 5×10^5 CD45.1⁺ (competitor) WT cells were injected into lethally irradiated (9 Gy, split dose) BL6-SJL (CD45.1⁺) mice. For "wt into mutant" experiments, wildtype congenic BL6/SJL (CD45.1⁺) bone marrow cells (1×10^6 cells/recipient) were transplanted into lethally irradiated 4 week old *OCD^{fl/+}* and *OCD^{fl/fl}* (CD45.2⁺) recipients. For "mutant into wt" experiments, *OCD^{fl/+}* or *OCD^{fl/fl}* (CD45.2⁺) were transplanted into lethally irradiated 4 week old BL6/SJL(CD45.1⁺) animals. Donor cell engraftment was confirmed by FACS.

Full methods and any associated references are available as supplementary information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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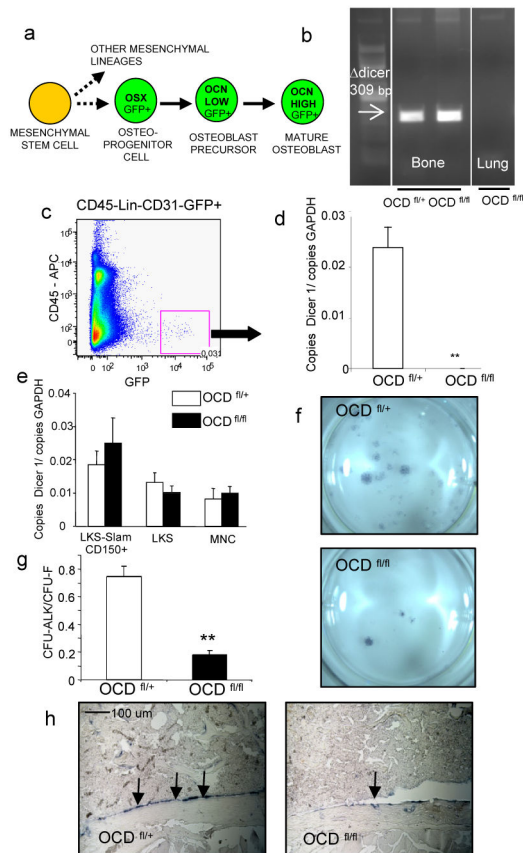


Figure 1. Impaired osteoblastic differentiation in OCD fl/fl mice

a, endogenous osterix expression in osteolineage cells. *Dicer1* deletion was demonstrated by **b**, genomic PCR and **c,d**, gene expression of primary osteolineage cells ($n=5$) **e**, *dicer1* gene expression in hematopoietic subsets excluding *dicer1* deletion from hematopoietic cells in OCD fl/fl mice ($n=4$) **f-h** impaired osteogenic differentiation capacity of OCD fl/fl bone marrow stromal cells as shown by reduced CFU-Alk (**f,g**) ($n=2$, performed in quadruplicate) and decreased *osteocalcin* gene expression by *in situ* hybridization (**h**). Data are mean \pm s.e.m. * $p < 0.05$, ** $p < 0.01$. OSX=osterix, LKS=lineage⁻ckit⁺Sca⁻ cells, CFU-ALK=colony forming unit alkaline phosphatase. For further details see supplementary information.

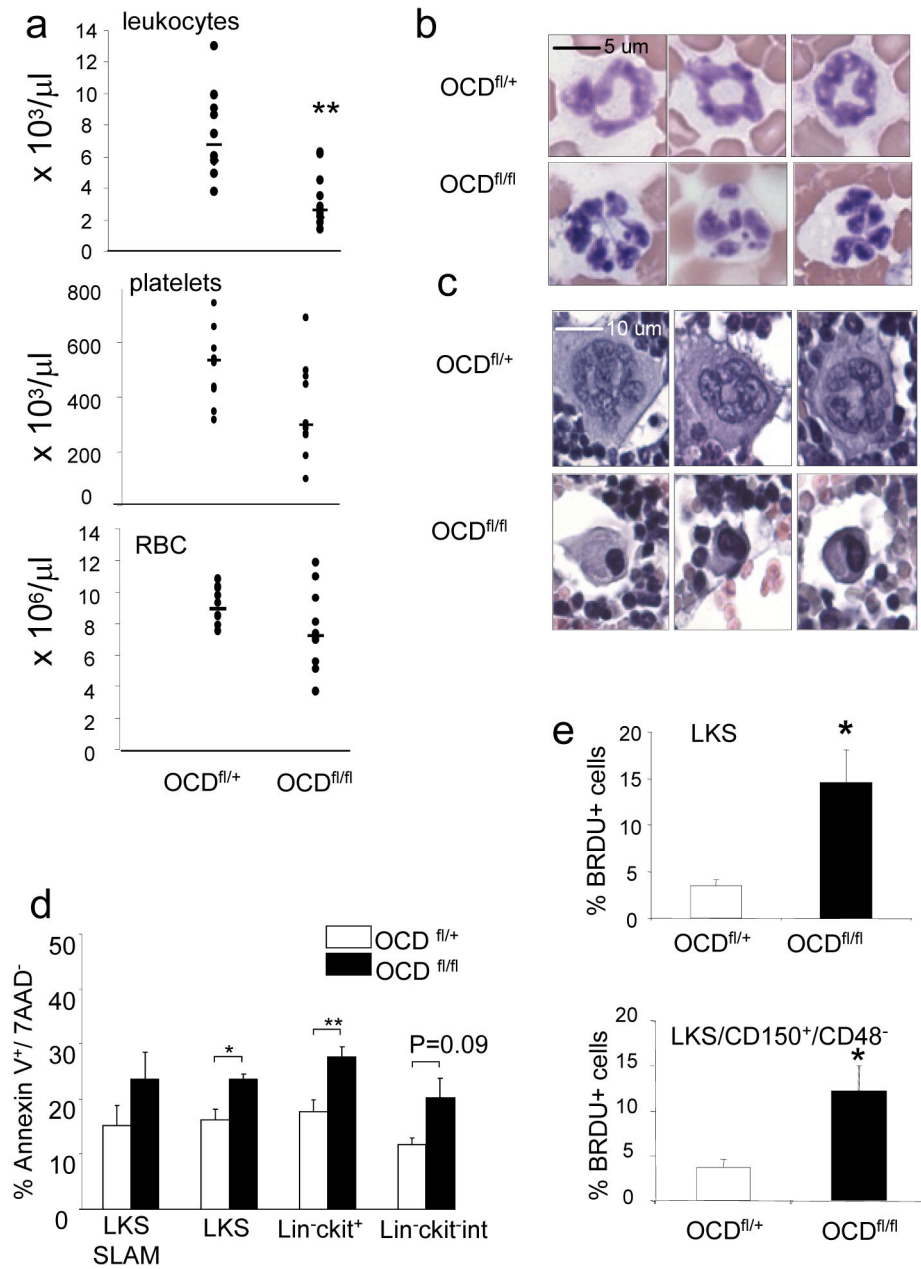


Figure 2. Myelodysplasia in OCD^{fl/fl} mice

a, Leukopenia with variable anemia ($p=0.16$) and thrombocytopenia ($p=0.08$) in OCD^{fl/fl} mice ($n=10$). **b**, blood smears showing dysplastic hyperlobulated nuclei in granulocytes **c**, bone marrow sections showing micro-megakaryocytes with hyperchromatic nuclei **d**, increased apoptosis of hematopoietic progenitor cells in OCD^{fl/fl} mice. ($n=4$) **e**, increased proliferation of hematopoietic progenitor cells as shown by in vivo BRDU labeling ($n=4$). Data are mean \pm s.e.m. * $p < 0.05$, ** $p < 0.01$. RBC=red blood cells, LKS= lineage⁻C-kit⁺ Sca1⁺ cells LKS-SLAM= lineage⁻C-kit⁺ Sca1⁺ CD150⁺ CD48⁻ cells L-K+= lineage⁻C-kit⁺ cells L-K-int=lineage⁻Ckit^{intermediate}, BRDU= bromodeoxyuridine. For further details see supplementary information.

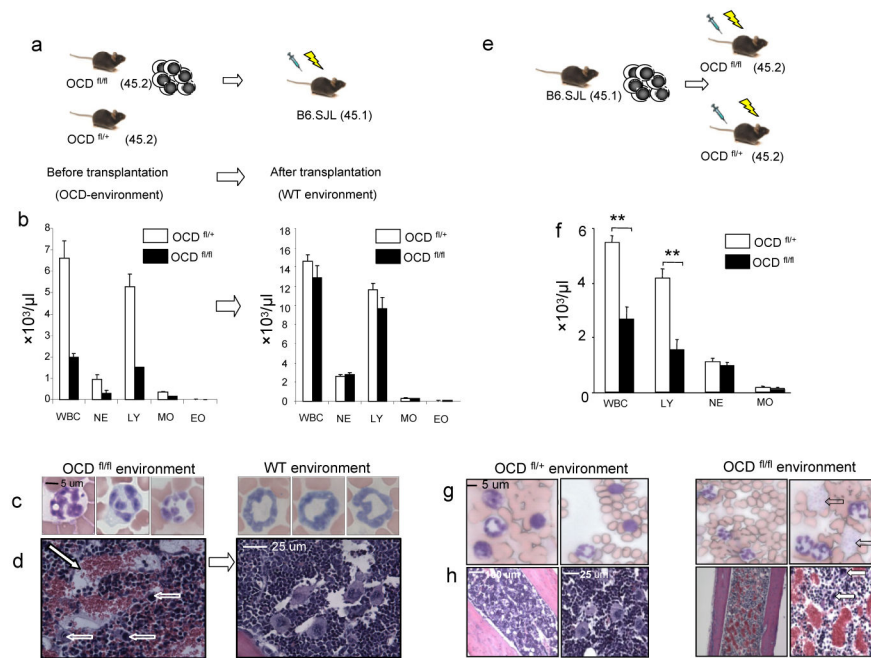


Figure 3. Myelodysplasia in OCD fl/fl mice is induced by the bone marrow microenvironment
a, bone marrow cells of OCD fl/fl or littermate OCD $fl/+$ mice ($n=2$) were transplanted into lethally irradiated WT (B6.SJL) mice ($n=4$ per OCD mouse) demonstrating complete normalization of leukopenia (**b**), granulocyte (**c**) and megakaryocyte (**d**, open arrows) morphology (open arrows) and bone marrow vascularity (**d**, closed arrow) 16 weeks post-transplant. Conversely, when **e**, wildtype B6.SJL cells were transplanted into OCD $fl/+$ ($n=8$) or OCD fl/fl ($n=8$) mice, hematopoiesis in mutant mice at 8 weeks displayed **f**, leucopenia **g**, dysgranulopoiesis with giant platelets (indicated by arrows) and **h**, increased bone marrow vascularity with dysplastic megakaryopoiesis (arrows). Data are mean \pm s.e.m. * $p < 0.05$, ** $p < 0.01$. For further details see supplementary information.

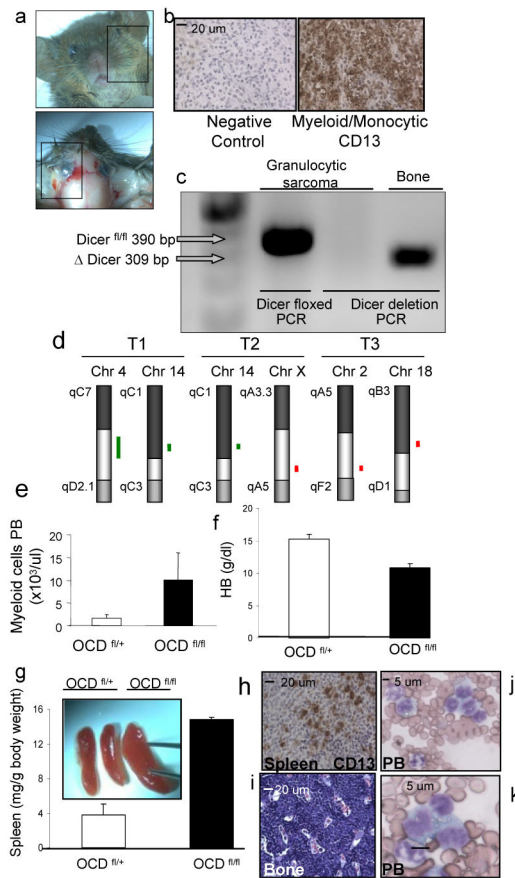


Figure 4. Myeloid sarcoma and acute myelogenous leukemia in *OCD^{fl/fl}* mice
a, tumors infiltrating soft tissue in 2–4 weeks old mice. Data from one representative animal. **b**, tumor sections showing predominantly myeloblasts admixed with maturing granulocytes, typical of myeloid sarcoma and confirmed by immunostaining with CD13. **c**, exclusion of *Dicer1* deletion in myeloid sarcoma cells by genomic PCR. Bone from the same animal as positive control. **d**, genomic aberrancies detected in myeloid sarcomas (n=3) as detected by array-based CGH. A common amplified region was identified on chromosome 14qC1 in two tumors. Green lines represent amplifications, red line represents deletion **e**, myelocytosis, **f**, anemia, **g**, splenomegaly with **h**, infiltration of CD13⁺ blasts in the spleen and **i**, bone marrow, and **j–k**, monocytic blasts in a background of dysplastic granulocytes in the peripheral blood. HB=hemoglobin, PB=peripheral blood

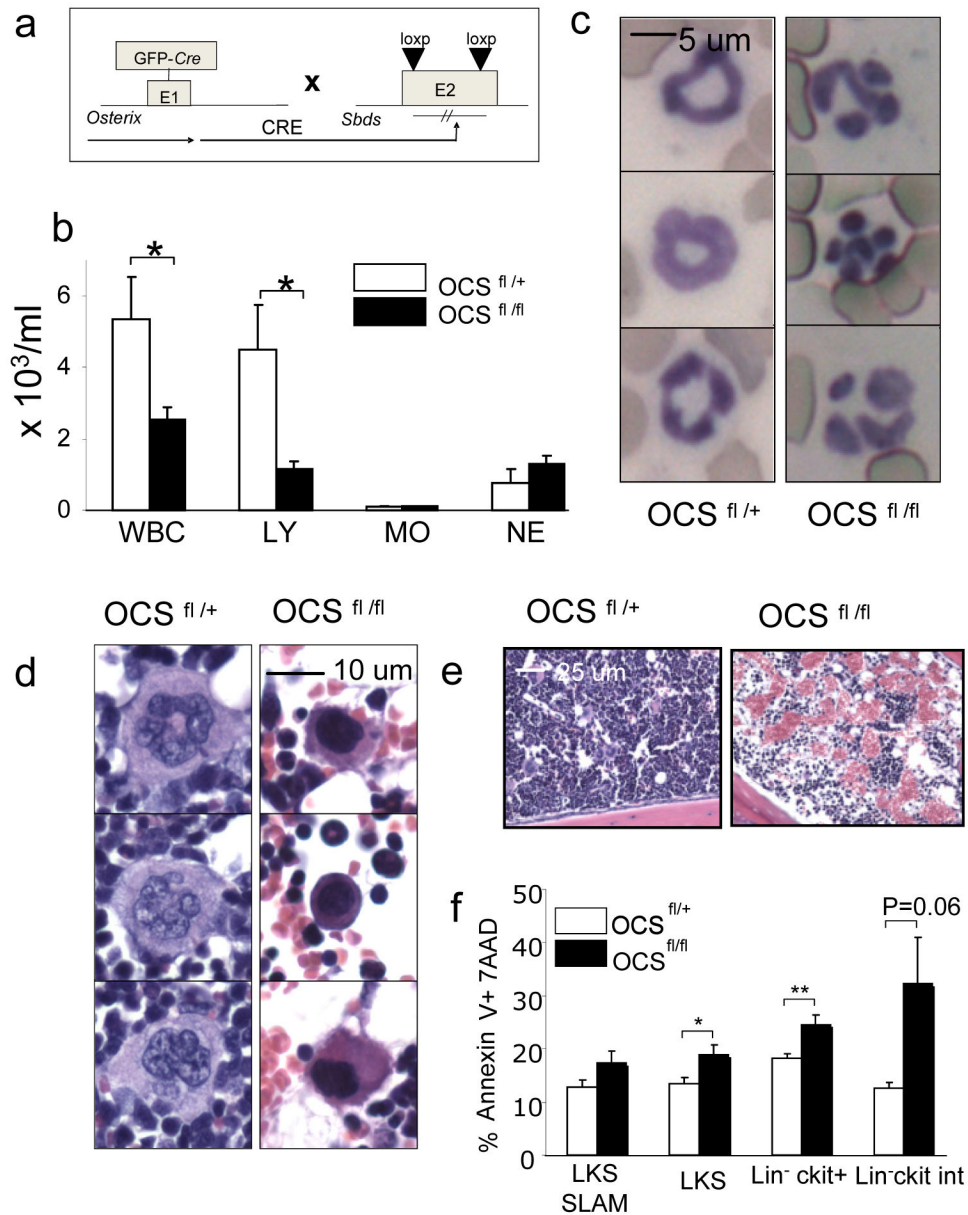


Figure 5. Targeted deletion of the *Sbds* gene from osteoprogenitor cells recapitulates many features of the OCD fl/fl phenotype

a, genetic model of *Sbds* deletion from osteoprogenitor cells **b**, leukopenia (n=8) **c**, dysplasia of neutrophils in peripheral blood **d**, dysplasia of megakaryocytes (micro-megakaryocytes) **e**, hypervascularity of the bone marrow **f**, increased intramedullary apoptosis of hematopoietic progenitor cells (n=5). Data are mean \pm s.e.m. * p 0.05, **p 0.01.