A novel mouse model provides insights into the neutropenia associated with the ribosomopathy Shwachman-Diamond syndrome

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Ribosomopathies are rare diseases caused by mutations in proteins constituting the ribosome (ribosomal proteins) or factors involved in ribosome production (ribosome biogenesis factors). With the exception of 5q- syndrome, which is an acquired disease, most established ribosomopathies are congenital diseases. The most frequent congenital diseases are Diamond-Blackfan anemia (DBA), Shwachman-Diamond syndrome (SDS) and Treacher Collins syndrome (TCS), each having an incidence that ranges from 1 in 50,000 to 1 in 200,000 live births. Ribosomopathy patients typically present with developmental abnormalities. Although all ribosomopathies are caused by defective ribosome function, these abnormalities can manifest under various forms, such as hematopoietic defects (e.g. anemia and other cytopenias), craniofacial malformations, short stature, mental and motor retardation. Some ribosomopathies have also been reported to be at increased risk of developing cancer. For example, DBA and SDS patients are predisposed to develop acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), and DBA patients also tend to have an elevated risk of solid tumors.[1,2]

The field of ribosomopathy is currently challenged by several unanswered questions. First, we do not understand how disruption of the ribosome, an organelle considered to have a generic role in all tissues, can cause such a diverse spectrum of clinical presentations with typical tissue-specific pathologies observed for each ribosomopathy. Second, the molecular mechanisms leading from a ribosome biogenesis defect towards the developmental abnormalities is poorly understood. Many groups have published data supporting activation of the tumor suppressor protein p53 (TP53) in response to a biogenesis defect, which then leads to cell cycle arrest and apoptosis, and may cause the developmental defects.[3] However, TP53-independent mechanisms have been described that may also contribute to these phenotypes.[4] Finally, the symptoms in ribosomopathy patients show an intriguing paradox: initially, these patients present developmental defects linked to apoptosis and cellular hypoproliferation while later in life, they are predisposed to develop cancer, a typical disease of resistance to cell death and cellular hyperproliferation. Attractive models that may explain this transition have been proposed.[5]

The key towards answering these questions is the development of good animal models that reproduce the clinical pathology of the human patients. However, this is often not as easy as it sounds. Mouse and zebrafish are the two model organisms that have been exploited most to model ribosomopathies. Whereas zebrafish offers the advantage of allowing relatively fast genetic manipulation and easy *in vivo* imaging of the hematopoietic system, mouse models have other advantages, such as the availability of a larger toolbox of molecular biology reagents.[6] Heterozygous loss-of-function defects in the *RPS19* gene are found in 25% of DBA cases, making it the most common DBA causative gene. The zebrafish morpholino knockdown model of the *rps19* gene displays severe anemia due to reduction of erythrocytes, as well as morphological abnormalities, such as a malformed brain and a curved tail.[7] Several groups have made attempts to model the DBA phenotype in *Rps19* knock-out and knock-in mice. Initially, this was without much success and, at best, only mild anemia phenotypes could be induced.[8] Recently, an inducible RNA interference mouse model was described. This is the mouse model that currently most closely resembles human phenotypes, and these *Rps19*-deficient mice develop a macrocytic anemia together with leukocytopenia, variable platelet count, and later bone marrow exhaustion and failure.[9] Also for SDS, attempts have been made to generate animal models that mirror the human pathology. Approximately 90% of patients with a clinical diagnosis of SDS carry biallelic inactivating mutations in the *SBDS* gene.[10] SBDS is a protein with a well-documented role in the later steps of ribosome biogenesis. In particular, SBDS interacts with the GTPase EFL1 to catalyze the removal of eIF6 from the 60S ribosomal subunit. Removal of eIF6 is a prerequisite for the association of the 60S and with the 40S subunit, and thus for the formation of an actively translating ribosome.[11,12] SBDS is, however, a multifunctional protein, and several non-ribosomal roles, which might also be relevant for its disease-causing action, have been described, such as a role in mitotic spindle stabilization.[13] Clinically, SDS presents with exocrine pancreatic

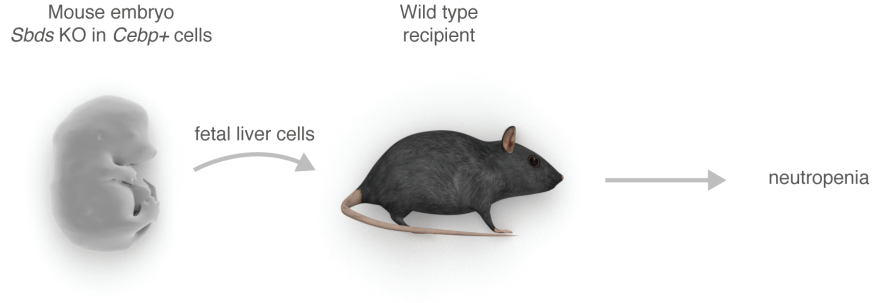

Figure 1. Schematic representation of the mouse model for SDS associated neutropenia described by Zambetti *et al.*[18] Fetal liver cells from E14.5 embryos with Sbds deficiency in Cebpa-expressing cells (*Sbds*$^{f/f}$; *Cebpa*$^{Cre/+}$; *R26*$^{EYFP/+}$) are collected and injected into wild-type B6.SJL mice, leading to neutropenia development in the recipient mice. The mouse embryo cartoon was kindly provided by the e-mouse atlas project (http://www.emouseatlas.org)

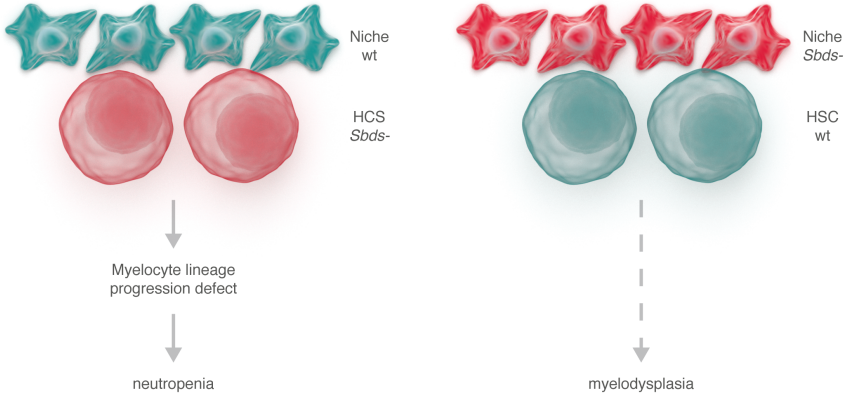

Figure 2. Wild-type mice transplanted with Sbds deficient HSCs develop neutropenia (left). Mice with Sbds inactivation in the niche (osteoprogenitor cells) develop myelodysplasia (right).

insufficiency, ineffective hematopoiesis (with neutropenia as the most predominant problem), skeletal and cognitive defects, and an increased risk of neoplastic transformation, particularly to MDS and AML.[14] Morpholino knockdown of the *sbds* ortholog in zebrafish nicely recapitulates the human syndrome, with the fish displaying pancreatic hypoplasia, loss of neutrophils, and skeletal defects.[15] So far, mouse modeling of SBDS has been challenging. Complete knockout of *Sbds* is lethal to mice[16] and transplant of mouse hematopoietic stem cells (HSCs) with shRNA knockdown of *Sbds* into wild-type recipients causes impaired myeloid progenitor generation but no overt neutropenia.[17] In this issue, Raaijmakers and his team describe an elegant novel mouse model for SDS.[18] They made use of conditional *Sbds* knock-out mice and crossed them to mice expressing the CRE recombinase in Cebpa positive cells, with the aim of inactivating *Sbds* in a subset of HSCs. Whereas deficiency of *Sbds* in all *Cepba*-expressing tissues turned out to be lethal, embryos with *Sbds* deficiency in *Cepba*-positive cells were healthy at gestational age and showed normal fetal liver architecture. Therefore, this allowed *Sbds*-deficient fetal liver cells from these embryos to be collected and transplanted into irradiated wild-type recipients (Figure 1). Recipient mice developed hypocellular bone marrow (mainly due to a reduction of neutrophils) and a profound neutropenia in the peripheral blood. Interestingly, only the more mature myeloid cells beyond the stage of myelocytes and metamyelocytes (MC-MMs) showed reduced cell numbers in this model, and an expansion of myeloid progenitor cells up to the MC-MM stage was found. Further characterization of the model revealed a lineage progression block in the myelocytes due to failure to exit the cell cycle, a critical requirement for terminal differentiation of myelocytes into mature neutrophils. This myelocyte differentiation block is also supported by downregulation of myeloid transcription factor RARα and its target genes, as well as reduced expression of genes encoding secondary and tertiary granules in the transcriptomes of *Sbds*-deficient MC-MMs (Figure 2). Finally, the authors show that the *Sbds*-deficient MC-MMs show activation of TP53, suggesting that cellular stress-induced activation of TP53 and associated apoptosis may explain at least part of this phenotype. Whereas in this way the recipients of *Sbds*-deficient HSCs nicely recapitulated the neutropenia phenotype of human SDS, the myelodysplasia, which is another hematologic hallmark of SDS, was not observed in this model.[18] Neither was leukemic transformation seen, though longer follow up of the animals than the reported four months may be required in order to observe such phenotypes. Interestingly, Raaijmakers *et al.* showed previously that deletion of *Sbds* in osteoprogenitors, cells that form the niche for HSCs, induces bone marrow dysfunction with myelodysplasia in mice.[19] This suggests that the MDS phenotype in SDS may be driven by cell-extrinsic factors (Figure 2). It would now be interesting to test if transplantation of *Sbds* knock-out HSCs into mice with Sbds-deficient osteoprogenitors would reproduce both the SDS-associated neutropenia and MDS in mice.

The new SDS disease model does provide some novel clues to answer the questions surrounding ribosomopathies, such as the tissue-specificity of disease phenotypes. It shows that, whereas rapidly cycling progenitors can deal with loss of *Sbds* function, myelocytes show a particular dependency on *Sbds* for their maturation into neutrophils; what the molecular biological mechanisms behind this observation are still have to be determined. In the context of DBA, it has recently been shown that erythroid cells with ribosomal protein deficiencies have a reduced translation efficiency of GATA1,[20] a transcription factor with an essential role in the formation of erythroid cells, megakaryocytes and eosinophils from hematopoietic stem and progenitor cells. *Sbds* deficiency does alter translation and ribosome biogenesis-related gene sets in MC-MMs.[18] This suggests that ribosome function in these cells could be altered, and that translation of factors essential for myelocyte differentiation could potentially be impaired. The mouse model described by Zambetti *et al.* will allow these mechanistic aspects to be investigated in more detail, which over the longer term could result in the development of targeted therapies that can correct the morbidity and mortality due to SDS-associated neutropenia.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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