to remarkable advances in disease modeling and regenerative medicine in the future.

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Resistance in the Ribosome: RUNX1, pre-LSCs, and HSPCs

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Therapeutic targeting of pre-leukemic stem cells (pre-LSCs) may be a viable strategy to eradicate residual disease and prevent leukemia relapse. Now in *Cell Stem Cell*, Cai et al. (2015) show that loss-of-function mutations in *RUNX1* reduce ribosome biogenesis and provide pre-LSCs a selective advantage over normal hematopoietic cells through increased stress resistance.

The myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal bone marrow malignancies characterized by ineffective hematopoiesis, the presence of dysplastic cells in the bone marrow, and peripheral blood cytopenias. MDS occurs more frequently in older males and in individuals with prior exposure to cytotoxic therapy (Garcia-Manero, 2012), and individuals with MDS have an increased risk of developing acute myeloid leukemia (AML) (Heaney and Golde, 1999). Recent experimental evidence suggests that MDS arises from a series of transforming events that accumulate to generate pre-leukemic stem cells (pre-LSCs), the precursors of fully transformed LSCs (Pandolfi et al., 2013). Transformational genetic and epigenetic changes are believed to selectively expand pre-LSCs in the bone marrow, which then out-compete normal hematopoietic stem and progenitor cells (HSPCs). Genome-wide studies have recently identified a number of genetic lesions that are implicated in this process and the development and/or progression of MDS. These lesions have so far been found in splicing factor genes (e.g., SF3B1 and SRSF2) as well as genes involved in regulating DNA methylation (e.g., TET2, IDH, and DNMT3A), histone modification (e.g., ASXL1 and EZH2), and several signal transduction and transcription factors (e.g., RUNX1, p53, EVI1, JAK2, and FLT3). In this issue of *Cell Stem Cell*, Cai et al. (2015) show that mutations in the transcription factor RUNX1 reduce ribosomal biogenesis and provide a competitive advantage to pre-LSCs by enhancing stress resistance.

Almost half of MDS patients present with recurring karyotypic abnormalities affecting chromosomes 5, 7, 8, and 20, many of which impact the ribosome. Hemizygous loss of the ribosomal protein gene *Rps14* contributes to the development of anemia in 5q$^-$ syndrome (Ebert et al., 2008). Nucleophosmin, which is located on chromosome 5q35.1, has been implicated in MDS pathogenesis and is also critical for ribosome function (Grisendi et al., 2006; Reschke et al., 2013). Other genetic abnormalities cause impaired ribosome biogenesis (Ribi) and function—a collection of disorders known as ribosomopathies. Researchers have also found an association between ribosomal stress and activation of p53. In their current study, Cai et al. have focused on *Runx1*, a DNA binding transcription

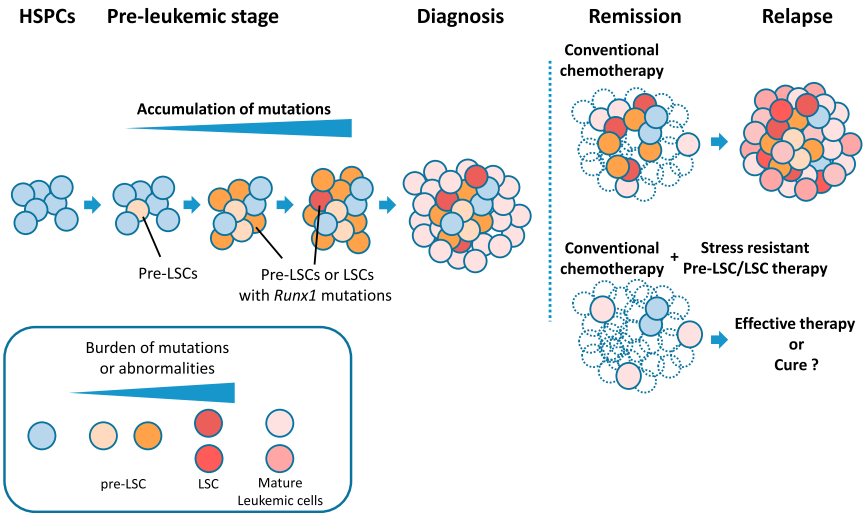

Figure 1. Hypothetical Development of Therapeutic Strategies Targeting Stress-Resistant pre-LSCs and/or LSCs

Loss of function of *RUNX1* mutations in HSPCs can be early or later events in the progression of MDS or AML, and these mutations can provide pre-LSCs with selective advantages over normal HSCs. Determining the precise mechanisms of survival and stress resistance in these cells may lead to the development of combination therapies to eradicate leukemic cells.

factor that is found mutated in MDS and AML, particularly in patients with previous exposure to genotoxic agents. In the mouse, loss-of-function mutations of *Runx1* cause defects in lymphocyte and megakaryocytic development (Cai et al., 2011). Intriguingly, deficiency of *Runx1* alone only minimally impacts long-term hematopoietic stem cells (LT-HSCs) (Cai et al., 2011), while *Runx1*; *Runx3* double-knockout mice exhibit lethal phenotypes due to bone marrow failure and myeloproliferative disorder (Wang et al., 2014). Early events such as *RUNX1* mutations are known to generate pre-LSCs. However, the molecular mechanisms underlying the competitive expansion of pre-LSCs through loss-of-function *RUNX1* mutation have yet to be fully understood.

Cai and colleagues now elucidate some of the precise machinery involved in this process. Using conditional *Runx1*-deficient mice, they first found that *Runx1* deficiency protects HSPCs from various stresses. *Runx1*-ablated HSPCs expanded in the bone marrow relative to competitor cells, when donor cells were subjected to a low level of irradiation prior to transplantation. Less apoptosis was observed in $Runx1^{\Delta/\Delta}$ HSPCs after Ara-C treatment and endoplasmic reticulum (ER) stress induced by tunicamycin. Based on these data, Cai et al. concluded that *Runx1*-deficient HSPCs are resistant to both genotoxic and endogenous stresses. *Runx1*-deficient HSPCs are slow cycling and have a low metabolic profile, small cell phenotype, and decreased biosynthetic capacity with a balanced reduction in Ribi. To understand the mechanisms underlying decreased Ribi, they analyzed ChIP-seq data generated in human $CD34^{+}$ HSPCs and found that RUNX1 binding is highly enriched at the promoters of Ribi genes. In murine $Runx1^{\Delta/\Delta}$ HSPCs, expression levels of ribosome genes occupied by RUNX1 were reduced. Acute deletion of *Runx1* in vitro decreases 45S rRNA and the translational rate in HSPCs. These data suggest that RUNX1 directly regulates Ribi through its enriched binding at the promoters of Ribi genes, including the genes encoding structural components of the ribosome. Interestingly, *Runx1*-deficient HSPCs have lower levels of p53 protein and reduced apoptosis. Increased levels of p53 or its target genes by radiation were also attenuated in $Runx1^{\Delta/\Delta}$ HSPCs. Activation of p53 alone fails to reverse the low apoptotic phenotype in *Runx1*-deleted HSPCs, and increased mTOR signaling partially restores Ribi, but not their reduced apoptotic phenotype.

While these findings represent a step forward in developing a coherent picture of the competition between pre-LSCs and HSPCs and how this may lead to full-blown malignancies, many gaps remain in this developing story. Perhaps Cai et al.'s most compelling new findings are the links demonstrated between *Runx1* mutations, reduced Ribi, and p53-independent stress resistance phenotypes. These findings, however, raise a series of theoretical issues. One such question is the mechanism of how the changes in Ribi induced by *Runx1* mutations lead to stress resistance in HSPCs. In other words, is this phenomenon simply the result of slow growth resulting from *Runx1* deficiency, or do other mechanisms contribute specifically in the case of reduced Ribi? It will also be interesting to explore which ribosome genes are major players in Ribi phenotypes induced by *Runx1* mutations.

For example, are one or a few members of the perturbed ribosome genes able to restore the phenotype of *Runx1*-mutated HSPCs? Located on chromosomal 21, the *RUNX1* gene is also involved in chromosomal translocations in leukemia, and the RUNX1-ETO fusion protein by t(8;21) is one of the most common translocations in AML (Lam and Zhang, 2012). It will also be interesting to unravel whether Ribi contributes to leukemia pathogenesis induced by RUNX1 fusion proteins. The answer to these questions will no doubt be the focus of future studies, which will lead to a deeper characterization of these mechanisms, such as specific downstream targets, and the development of new therapeutic approaches designed to eradicate stress-resistant leukemic and pre-leukemic HSPCs while restoring normal hematopoiesis (Figure 1). As ribosomopathies have also been associated with an increased predisposition to cancer, these findings may have wide-ranging implications in other cancers.

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Cell Cycle Rules Pluripotency

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Stem cell self-renewal is intrinsically associated with cell cycle control. However, the precise mechanisms coordinating cell fate choices and cell cycle remain to be fully uncovered. Now in *Cell*, Gonzales et al. (2015) and colleagues demonstrate that factors controlling the G2/M phase are necessary to block pluripotency upon induction of differentiation.

Stem cells are defined by their ability to proliferate almost indefinitely while maintaining their capacity to differentiate into several cell types. The coordination of these two properties, self-renewal and multipotency, is essential to ensure proper embryonic development, organogenesis, organ homeostasis, and tissue repair upon injury. Furthermore, uncontrolled proliferation of stem cells could play a major role in diseases such as cancer. Thus, understanding the interplay between cell-cycle regulation and cell fate decisions represents a major interest for the stem cell field. Nonetheless, the study of these mechanisms has been restricted by the technical difficulties impairing investigations of cell-cycle regulations in vivo and also by the lack of appropriate in vitro model systems. Now in *Cell*, Huck-Hui Ng and colleagues demonstrate that pluripotency of human embryonic stem cells (hESCs) is controlled by factors necessary for the transition of the G2/M phase of the cell cycle. This study provides new insights into the mechanisms by which stem cells exploit cell-cycle machinery to control their cell fate decisions (Gonzales et al., 2015).

The cell cycle can be divided into four different phases: the G1 phase, during which a cell decides to engage in a new division; the S phase, when DNA is replicated; the G2 phase, which allows DNA repair mechanisms; and the M phase, at the end of which cells divide. The G1 phase has been the focus of a broad number of studies on stem cells since cell fate choices seems to occur or at least be initiated during this part of the cell cycle. Indeed, several reports have shown that stem cells can perceive differentiation signals specifically in G1. Of particular interest, the early G1 phase is permissive for endoderm differentiation in hESCs while the late G1 phase is only permissive for neuroectoderm specification (Pauklin and Vallier, 2013). This divergent capacity of differentiation is established by CyclinD/CDK4-6, which are expressed during the late G1 phase. These cell-cycle regulators inhibit the Activin/Nodal signaling pathway, which is known to block neuroectoderm differentiation of hESCs while being necessary for endoderm differentiation. Importantly, the importance of the G1 phase and CyclinD/CDK4-6 appear to be conserved in adult stem cells (Lange et al., 2009; Mende et al., 2015). Thus, the cell-cycle machinery could directly orchestrate initiation of differentiation during the G1 phase progression in a diversity of cell types.

The importance of S/G2 in stem cell control is by far less explored. Regulators of these phases of cell cycle such as Cyclin B1 are necessary for cell survival and their absence often results in cell death and/or major genomic anomalies. For these reasons, the importance of the G2 phase in stem cell self-renewal and differentiation remains to be fully uncovered. The report by Gonzales et al. (2015) remedies this shortfall by revealing that the G2 transition could also have a key function in the mechanisms directing hESC differentiation.

The authors first performed an siRNA screen to identify factors that could delay differentiation induced by the absence or inhibition of TGFβ/Activin/Nodal and FGF signaling, both of which are known to be essential for the pluripotent state of hESCs (Thomson et al., 1998; Vallier et al., 2004). This screen revealed that several epigenetic mechanisms such as histone acetylation and chromatin remodeling are essential for the transition between pluripotency and differentiation to