tropenia and mutations in G6PC3. N Engl J Med 2009;360:32-43.
22. Rake JP, ten Berge AM, Visser G, Verlind E, Niezen-Koning KE, Buys CH, et al. Glycogen storage disease type Ia: recent experience with mutation analysis, a summary of mutations reported in the literature and a newly developed diagnostic flow chart. Eur J Pediatr 2000 May;159:322-30.
23. Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia Roncarolo M. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. Blood 2009;113:6288-95.
24. Thrasher AJ. WASp in immune-system organization and function. Nat Rev Immunol 2002;2:635- 46.
25. Ancliff PJ, Blundell MP, Cory GO, Calle Y, Worth A, Kempski H, et al. Two novel activating mutations in the Wiskott-Aldrich syndrome protein result in congenital neutropenia. Blood 2006;108:2182-9.
26. Beel K, Cotter MM, Blatny J, Bond J, Lucas G, Green F, et al. A large kindred with X-linked neutropenia with an I294T mutation of the Wiskott-Aldrich syndrome gene. Br J Haematol 2009;144:120-6.
27. Devriendt K, Kim AS, Mathijs G, Frints SG, Schwartz M, Van Den Oord JJ, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 2001;27:313-7.

Defective ribosome biogenesis in myelodysplastic syndromes

Naomi Galili, Samir Ahmed Qasim, and Azra Raza

Saint Vincent's Comprehensive Cancer Center, New York, NY, USA. E-mail: araza@aptiumoncology.com
doi:10.3324/haematol.2009.012021

Malignant disorders are poorly understood at the biological level in general, and myelodysplastic syndromes (MDS), a heterogeneous clonal stem cell disorder, in particular, are further confounded by representing a group of diseases rather than a single entity with clinical and biological heterogeneity within each subtype. The basis for grouping these assorted diseases under one MDS umbrella is the clinical presentation of variable cytopenias despite a generally cellular and dysplastic marrow. Diagnosis is based on bone marrow morphology, percentage of blasts, unexplained cytopenias and cytogenetics. These components have been used to classify the disease (French-American British[1] or FAB and World Health Organization[2] or WHO classifications) and to predict prognosis (International Prognostic Scoring System[3] or IPSS). The typical patient is elderly; thus with the increase in our aging population, the incidence of MDS has now surpassed that of acute myeloid leukemia (AML). Approximately 30% of MDS patients will progress to AML, but the majority die from infection or bleeding due to an increasing profundity of the cytopenias. The paradox of MDS is that despite peripheral cytopenia, the marrow is most often hypercellular. The initial breakthrough in understanding the biological basis of this paradox came with the demonstration that, in MDS, bone marrow cells were not only rapidly proliferating, but also undergoing excessive apoptosis.[4-7] As a result, the maturing hematopoietic cells are eliminated in the marrow and do not reach the peripheral blood, accounting for the cytopenias in the presence of a cellular marrow composed of mostly apoptotic cells. Furthermore, it was demonstrated that multiple cytokines involved in mediating apoptosis and proliferation are deregulated in MDS marrows.[7-9] While the MDS cell itself probably contributes, recent insights suggest that it is the bone marrow microenvironment that is also responsible for potentiating disease pathology and progression through cytokine imbalance. In summary then, MDS appears to be a disease of both the *seed and the soil* (the cell and its microenvironment) where peripheral cytopenias appear to be the result of an increased proliferation-increased apoptosis in the clonal cells and deregulated proapoptotic and trophic cytokines in the marrow.

Approximately half the patients with MDS present with recurring cytogenetic abnormalities most commonly affecting chromosomes 5, 7, 8, and 20. These have been shown to greatly affect prognosis and survival.[3] Despite the presence of these well recognized karyotypic aberrations, genetic mutations that accompany them have not been well understood and, in fact, mutation in a specific biological pathway that is common to multiple MDS subtypes has not been identified. In short, apoptosis has remained the sole unifying biological characteristic of the disease.

Recently, however, a possible cohesive genetic explanation for this increased apoptosis has been taking shape. Initial hints began with the study of patients who present with the specific karyotype abnormality of del(5q) in one allele. This is the most common chromosomal abnormality (10-15%) in MDS with two regions of deletions in the long arm of chromosome 5.[10] The more telomeric deleted region (the commonly deleted region or CDR) is found in a subtype of MDS; the 5q- syndrome. Patients with 5q- syndrome rarely transform to AML and have long survival. The well mapped CDR,[10] a 1.5MB interval flanked by marker D5S413 and the gene *GLRA1*, contains 40 genes, including two ribosomal genes, *RBM22* and *RPS14*. While no mutation in the undeleted allele was found in any of these genes, Boultwood *et al.*[11] noted that their expression was down-regulated in patients with 5q- syndrome (haplo-insufficiency). They speculated that deregulation of these genes and the pathway that they were part of, could be the causative abnormality in 5q- syndrome patients. This idea was supported by previous findings that genetic defects of ribosomal genes were implicated in congenital anemias including dyskeratosis congenita,[12] cartilage-hair hypoplasia,[13] Diamond-Blackfan anemia[14] and Shwachman-Diamond syndrome.[15,16] The importance of *RPS14* haplo-insufficency as a causative event in 5q- syndrome was then shown by Ebert et al. using RNAi to selectively inhibit each of the 40 genes within the deleted region.[17] Suppression of *RPS14* in normal CD34$^+$ cells resulted in a 5q- syndrome like phenotype while forced expression in the hematopoietic cells of patients with 5q- syndrome rescued the phenotype. Additionally, expression of multiple genes associated with ribosome biogenesis was found to

be decreased in CD34+ cells from del(5q) patients when compared to that from refractory anemia (RA) patients with normal karyotypes, as well as in healthy controls.[18] Since the rapid proliferation of erythroid precursors requires continuous protein synthesis, erythropoiesis would be highly sensitive to disruptions in ribosome biosynthesis and may account for the severe anemia seen in 5q- syndrome patients. In contrast, megakaryocytes which proliferate at a slower pace can accommodate the 50% reduced gene dosage by accumulating the ribosomal proteins to continue proliferation, resulting in the well recognized clinical presentation of the 5q- syndrome with anemia and thrombocytosis.

Most recently, small deletions were detected in several ribosomal genes, including *RPS14*, in CD34+ cells of patients with non-del(5q) MDS, suggesting that deregulated ribosomal biogenesis may not be limited to del(5q) MDS.[19] The question that remains, however, is what accounts for the increase in apoptosis found in the MDS marrow? Studies have shown that deregulated ribosome biogenesis leads to increased expression of p53 in what is now referred to as the ribosomal stress response; this can trigger apoptosis in a cell lineage specific manner.[20] Thus a picture is emerging of a possible unified molecular basis for the universal feature of cytopenias in MDS due to deregulated ribosomal biosynthesis leading to activation of p53 and resulting excessive apoptosis.

In this issue, Czibere *et al.*[21] report findings of an interesting study aimed at establishing whether *RPS14* expression levels are deregulated in patients that have MDS without the 5q- deletion and whether expression levels are related to disease outcome. RPS14 is a universal structural protein of the 40S ribosomal subunit which is essential for the cleavage of the 30S precursor rRNA molecule into the 18SE/18S rRNA molecule. Haploinsufficiency in MDS with del(5q) leads to impaired processing of the 18S ribosomal RNA with subsequent disruption of the small 40S subunit. Its causal role in 5q- syndrome and deletion in other types of MDS prompted this group to examine RPS14 expression by quantitative RT-PCR in bone marrow CD34+ cells from 72 non-del(5q) MDS patients, 11 MDS patients with 5q deletions and 11 normal donors. They found that 72% of the non-del(5q) patients showed a decrease in RPS14 expression equal to that of those with the 5q deletion. When WHO classification was considered in this group, 42% of the patients with lower risk MDS had decreased expression of RPS14 in contrast to 93% of those with higher risk disease. Since 5q- syndrome patients have improved survival, the authors investigated whether the decrease in RPS14 expression in the non-del(5q) group could be of similar prognostic value. Separating the patients according to their IPSS subgroups, the overall survival for patients with high-risk or INT-2 was, as expected, unaffected by expression of RPS14. However, for patients with INT-1, there was a significant increase in survival for patients with low expression of RPS14 as compared to those with high expression. Patients with low expression had not reached median survival with 40 months of follow-up while those with high expression had a median survival of 25 months. Multivariate analysis showed that expression of RPS14 was an independent prognostic indicator for survival.

This study shows for the first time that a proportion of non-del(5q) patients have low RPS14 expression when compared to normal controls and that these levels are similar to that of 5q- syndrome MDS patients. The incidence varies when the patients are grouped according to the IPSS as low- or high-risk (see below). While the genetic source for low expression is not investigated, the levels of expression suggest that there is haploinsufficiency. The mechanism by which low RPS14 expression, whether by deletion or other suppression mechanisms, prolongs survival is not clear. While mutations in ribosomal proteins have been identified as the cause of several congenital forms of anemia, MDS is the first disease where this mutation is acquired in an early stem cell. It has been previously shown that ~25% non-del(5q) MDS patients who respond to lenalidomide have an expression signature that reflects a defect in erythropoiesis.[22] This signature is also found in patients with del(5q) MDS, who as a group are very sensitive to therapy with this drug. Ectopic expression of RPS14 in CD34+ cells from patients with 5q- syndrome restores the erythropoietic signature.[17] Thus it is not unreasonable to find that, as with 5q-syndrome, survival is prolonged when RPS14 expression is suppressed in lower risk patients, despite the fact that many of the clinical features are not shared between these two groups of patients.

MDS is primarily a disease in which a stem cell obtains a growth advantage and overwhelms the marrow with its progeny. The clone typically exhibits both rapid proliferation and increased apoptosis, both of which are tightly regulated by p53 family proteins. Disruption of ribosomal biogenesis has been clearly demonstrated in multiple systems to greatly perturb p53 signaling.[23] The resulting aberrant phenotypes have been shown to be both ribosomal-protein specific and cell lineage specific. p53 can be stabilized or degraded and both types of deregulation are associated with tumorigenesis. Decreased RPS14 expression, however, does not by itself appear to be associated with leukemic transformation. It is also difficult to explain how such a defect could provide a growth advantage that results in clonal expansion. It is important to remember, however, that the MDS cell is expanding in a marrow micro environment that is increasingly abnormal and thus it is not clear what constitutes a growth advantage under such conditions. Other mutations most likely contribute to the clonal expansion of these cells. Indeed the finding that 92% of patients at high risk of transforming to AML also have low expression of RPS14 supports the idea that other mutations are required for the cell to become insensitive to apoptotic signals and to rapidly proliferate with the blast phenotype of AML.

The story of disrupted ribosomal biogenesis in MDS began with a critical observation by Boultwood *et al.*[11] that the expression of two important ribosomal genes, *RBM22* and *RPS14*, was decreased in 5q- syndrome patients, gained a significant boost with the demonstration by Ebert *et al.*[17] that *RPS14* haplo-insufficiency causes the 5q- syndrome, and is now evolving rapidly with multiple reports describing defective ribosomal biogenesis in non-del(5q) MDS patients. The study by Czibere

et al.[21] in the current issue represents a significant step forward in this chain as it links biology to prognosis by showing that lower risk non-del(5q) MDS patients with *RPS14* haplo-insufficiency tend to have a prolonged survival. Be that as it may, the fact remains that defective ribosomal biogenesis, a previously unsuspected mechanism, is emerging as a surprise contender for the lead role in disrupting erythropoiesis in a variety of anemias. An entirely original subject of research has exploded on the MDS scene, one which links congenital and acquired anemias, and which is likely to produce exciting biological insights in the future, especially when combined with the emerging improved technology.

Azra Raza is Professor of Medicine at New York Medical College and Director of the MDS Center at St. Vincent's Comprehensive Cancer Center in New York.

Naomi Galili is Associate Professor of Medicine at New York Medical College and Director of the Bone Marrow Translation Laboratory at St. Vincent's Comprehensive Cancer Center in New York.

Samir Ahmed Qasim is a Research Associate in the Clinical Research office at St. Vincent's Catholic Medical Center in New York.

NG and AR contributed equally to this work which was supported by grants from The Partnership for Cure and the M. Night Shyamalan foundation.

References

1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982; 51:189-99.
2. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting--Airlie House, Virginia, November 1997. J Clin Oncol 1999;17:3835-49.
3. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079-88.
4. Raza A, Mundle S, Iftikhar A, Gregory S, Marcus B, Khan Z, et al. Simultaneous assessment of cell kinetics and programmed cell death in bone marrow biopsies of myelodysplastics reveals extensive apoptosis as the probable basis for ineffective hematopoiesis. Am J Hematol 1995;48:143-54.
5. Raza A, Gezer S, Mundle S, Gao XZ, Alvi S, Borok R, et al. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. Blood 1995;86:268-76.
6. Yoshida Y, Anzai N, Kawabata H. Apoptosis in myelodysplasia: a paradox or paradigm. Leuk Res 1995;19:887-91.
7. Raza A, Mundle S, Shetty V, Alvi S, Chopra H, Span L, et al. Novel insights into the biology of myelodysplastic syndromes: excessive apoptosis and the role of cytokines. Int J Hematol 1996;63:265-78.
8. Claessens YE, Bouscary D, Dupont JM, Picard F, Melle J, Gisselbrecht S, et al. In vitro proliferation and differentiation of erythroid progenitors from patients with myelodysplastic syndromes: evidence for Fas-dependent apoptosis. Blood 2002;99:1594-601.
9. Campioni D, Secchiero P, Corallini F, Melloni E, Capitani S, Lanza F, et al. Evidence for a role of TNF-related apoptosis-inducing ligand (TRAIL) in the anemia of myelodysplastic syndromes Am J Pathol 2005;166:557-63.
10. Boultwood J, Fidler C, Strickson AJ, Watkins F, Gama S, Kearney L, et al. Narrowing and genomic annotation of the commonly deleted region of the 5q syndrome. Blood 2002; 99:4638-41.
11. Boultwood J, Pellagatti A, Cattan H, Lawrie CH, Giagounidis A, Malcovati L, et al. Gene expression profiling of CD34+ cells in patients with the 5q- syndrome. Br J Haematol 2007;139:578-89.
12. Heiss NS, Knight SW, Vulliamy TJ, Klauck SM, Wiemann S, Mason PJ, et al. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. Nat Genet 1998;19:32-8.
13. Ridanpää M, van Eenennaam H, Pelin K, Chadwick R, Johnson C, Yuan B, et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia. Cell 2001;104:195-203.
14. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet 1999;21:169-75.
15. Boocock GR, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. Nat Genet 2003;33:97-101.
16. Austin KM, Leary RJ, Shimamura A. The Shwachman-Diamond SBDS protein localizes to the nucleolus. Blood 2005;106:1253-8.
17. Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008;450:335-40.
18. Pellagatti A, Hellström-Lindberg E, Giagounidis A, Perry J, Malcovati L, Della Porta MG, et al. Haploinsufficiency of RPS14 in 5q- syndrome is associated with deregulation of ribosomal- and translation-related genes. Br J Haematol 2008;142:57-64.
19. Sohal D, Pellagatti A, Zhou L, Mo Y, Opalinska JB, Alencar C, et al. Downregulation of Ribosomal proteins is seen in Non 5q- MDS. American Society of Hematology Annual Meeting 2008. Abstract 854.
20. McGowan KA, Li JZ, Park CY, Beaudry V, Tabor HK, Sabnis AJ, et al. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. Nat Genet 2008;40:963-70.
21. Czibere AG, Bruns I, Junge B, Singh R, Kobbe G, Haas R, et al. Low RPS14 expression is common in myelodysplastic syndromes without 5q- aberration and defines a subgroup of patients with prolonged survival. Haematologica 2009; 94:1453-5.
22. Galili N, Ebert BL, Tamayo P, Bosco J, Mak R, Pretz J, et al. An erythroid differentiation signature predicts response to lenalidomide in myelodysplastic syndrome. PLoS Med 2008;5:e35.
23. Vousden KH, Prives C. Blinded by the Light: The Growing Complexity of p53. Cell 2009;137:413-31.

Mutations of *NOTCH1*, *FBXW7*, and prognosis in T-lineage acute lymphoblastic leukemia

Charles G. Mullighan

St Jude Children's Research Hospital, Department of Pathology, Memphis, TN, USA.
E-mail: charles.mullighan@stjude.org. *doi:10.3324/haematol.2009.012047*

Acute lymphoblastic leukemia (ALL) is the commonest childhood malignancy,[1] and a major cause of morbidity and mortality from hematopoietic malignancies in adults. While the outcome of treatment for ALL in children is generally favorable, with cure rates exceeding 80%, this is not the case in adults, given that relapse occurs in the majority of patients and carries a poor prognosis. Moreover, improvements in the out-