Clonal evolution in myelodysplastic syndromes with isolated del(5q): the importance of genetic monitoring

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Myelodysplastic syndromes (MDS) with isolated deletion of chromosome arm 5q (del(5q)) have a good prognosis with low risk of transformation to acute myeloid leukemia (AML). Laboratory investigations typically demonstrate macrocytic anemia with preserved or increased platelet counts, and characteristic hypolobated megakaryocytes in the marrow (Figure 1A). Recent studies have provided significant insight into the pathogenesis of del(5q) MDS, and have demonstrated that additional mutations may alter the natural history of the disease.

Haploinsufficiency of key genes mediates the disease

Although the commonly deleted region (CDR) at chromosome band 5q32-33 was defined a number of years ago,[1] the gene(s) responsible for the characteristic phenotype of del(5q) MDS remained elusive (Figure 1B). An shRNA approach independently knocking down all 40 coding genes located within the commonly deleted region demonstrated that depletion of ribosomal protein S14 (RPS14) impairs erythropoiesis of human CD34$^+$ cells *in vitro*.[2] Haploinsufficiency of *RPS14* in mice results in macrocytic anemia and dyserythropoiesis.[3] Interestingly, multiple ribosomal genes are down-regulated in CD34$^+$ cells of patients with del(5q) MDS, which is consistent with the impaired erythropoiesis being a result of a ribosomal processing defect.[4] The resulting ribosomal stress activates the p53 pathway in the erythroid progenitors resulting in cell cycle arrest or apoptosis.[5] Consistent with this finding, crossing mice hemizygous for *RPS14* with p53-deficient mice rescues the progenitor cell defect.[3]

However, *RPS14* haploinsufficiency alone does not explain the megakaryocytic dysplasia and the tendency to thrombocytosis, nor the clonal dominance of del(5q) MDS cells. Examination of non-coding genes at 5q31-5q35 revealed reduced expression of miR-145 and miR-146a in marrow cells from patients with del(5q) MDS.[6] Depletion of these two microRNAs (miRNA) in mice results in variable neutropenia, thrombocytosis, and hypolobulated megakaryocytes with reduced endomitosis in the marrow. Mice transplanted with marrow depleted for miR-145 and miR-146 succumb to a myeloproliferative/leukemic disorder.[7] These two miRNAs target genes involved in the innate immune response pathway, including *TIRAP* (miR-145) and *TRAF6* (miR-146a). Transplantation of *TRAF6*-transduced bone marrow into wild-type mice recapitulated the hematologic phenotype seen with depletion of miR-145/miR-146a including progression to AML or bone marrow failure, suggesting that ectopic activation of innate immune signaling in the hematopoietic stem/progenitor population is a pathogenic feature of del(5q) MDS.

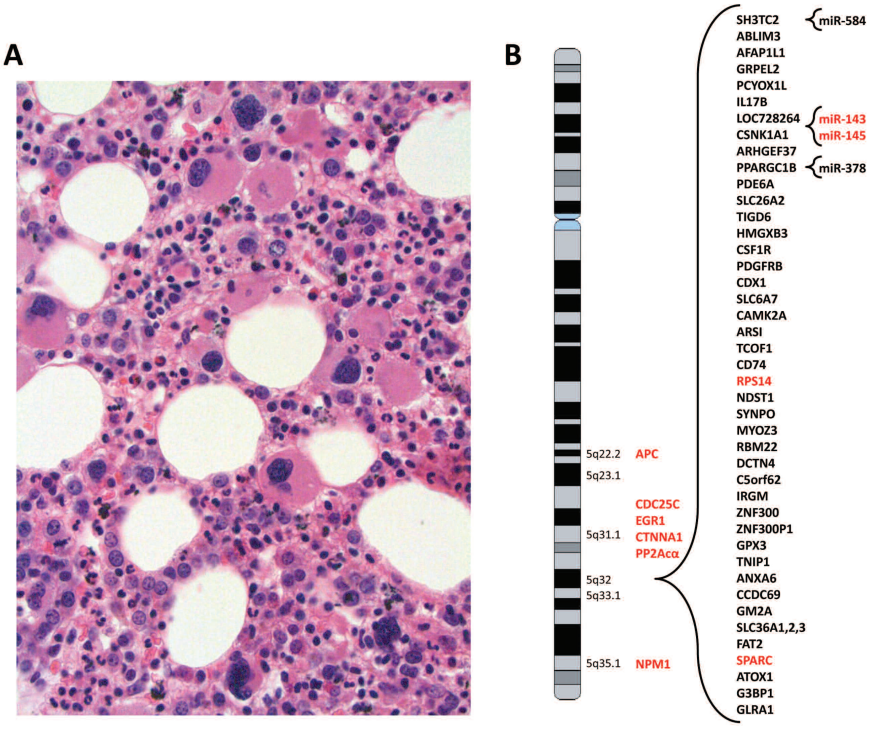

Figure 1. (A) Characteristic bone marrow morphology in MDS with isolated del(5q) showing numerous hypolobulated megakaryocytes. (B) All genes and micro-RNAs within the CDR at chromosome 5q32-33 are shown, and those implicated in the pathogenesis of the disease are highlighted in red. Genes located outside the CDR but associated with myelodysplasia, leukemic transformation, or the mechanisms of action of lenalidomide are also depicted in red. MDS: myelodysplastic syndromes, CDR: commonly deleted region.

Depletion of miR-145/miR-146a with activation of innate immune signaling results in NF-κB activation and upregulation of IL-6, which is also seen in patients with del(5q) MDS. The platelet and granulocytic defects driven by TRAF6-mediated activation of innate immune signaling and NF-κB are abrogated in mouse marrow cells lacking IL-6, but a similar proportion of mice still develop myeloid neoplasia.[6] Thus, while the paracrine effects of IL-6 likely explain the thrombocytosis and neutropenia, clonal dominance of the MDS cells in the marrow appears to be secondary to cell autonomous effects of miR-145/miR-146a haploinsufficiency and deregulated immune signaling.

Other genes on chromosome arm 5q have also been implicated in the pathogenesis of del(5q) MDS, including the tumor suppressor gene *SPARC* located within the commonly deleted region,[8] and others that are located outside the band 5q32-33 commonly deleted region associated with MDS, including *EGR1*, *CTNNA1*, *APC* and *NPM1* (Figure 1B).[9-11]

Altered bone marrow microenvironment and the effects of lenalidomide

Evidence suggests that there may be a bone marrow stromal defect in MDS with del(5q), resulting in impaired ability to support growth of normal hematopoietic progenitors. Intriguingly, treatment with lenalidomide reverses this deficiency, which is associated with an increase in SDF-1 and soluble ICAM 1.[12] It is conceivable that the del(5q) clone may alter the microenvironment, favoring the expansion of the malignant cells. Lenalidomide inhibits IL-6 and TNF-α, while inducing several other cytokines, and activates T cells and Natural Killer cells.[13] Thus, the favorable effects of lenalidomide on the stroma may be due to alterations in the cytokine profile in the marrow or indirectly via reduction of the malignant clone.

Other functions ascribed to lenalidomide include inhibition of the cell cycle regulating phosphatases *CDC25C* and *PP2Acα*.[14] Both these genes are located near the 5q32-33 commonly deleted region and are deleted in most del(5q) MDS patients (Figure 1B), potentially contributing to the increased sensitivity of del(5q) cells to lenalidomide. In addition, emerging data indicate that lenalidomide may increase miR-143 and miR-145 expression in $CD34^+$ del(5q) progenitors, and this induction may be associated with subsequent clinical response to treatment.[15] The tumor suppressor gene *SPARC* located within the 5q32-33 commonly deleted region is also up-regulated by lenalidomide.[8] The most important mechanisms for the potent effects observed in patients with del(5q) MDS must still be clarified.

Controversies regarding clinical management: the role of lenalidomide

According to international guidelines erythropoietic growth factors remain the first-line therapy in MDS with del(5q).[16] Patients not eligible for growth factor treatment due to high endogenous serum erythropoietin may require chronic transfusion therapy. 5-azacytidine is currently only recommended in higher risk MDS, although ongoing trials are evaluating its role in low-risk MDS. Allogeneic stem cell transplantation is not recommended in the absence of disease progression.[16]

Lenalidomide has unparalleled activity in transfusion-dependent del(5q) MDS patients, with 67% achieving transfusion independency and 45% complete cytogenetic remission.[17] This led to early approval by the Food and Drug Administration in the United States in 2005. However, the European Medicines Agency (EMEA) requested more data on optimal dosing and safety. While an extended study was ongoing, long-term follow up of the MDS-003 trial showed an unexpectedly high frequency of leukemic evolution. The outcome of the 42 German patients in the MDS-003 study has been published; 36% underwent AML transformation and 40% developed additional karyotypic abnormalities.[18] Subsequently, in 2008, the EMEA decided not to approve lenalidomide in del(5q) MDS due to safety concerns. It cannot be ruled out that the observed outcome of patients treated with lenalidomide reflects the natural course of the disease. The study patients had more advanced disease than the historical controls,[19] since all were transfusion-dependent and started treatment a median of 2.5 years after diagnosis. Moreover, the close monitoring during the study may have affected the recorded progression rate. International efforts aimed at resolving this issue need to adjust for multiple risk factors and the delayed treatment.

Genetic heterogeneity in del(5q) MDS

Until recently, no recurrent mutation had been described in MDS with isolated del(5q). A recent report described a del(5q) MDS patient who acquired a complex karyotype including del(17p13) where the potent tumor suppressor gene *TP53* is located. Sequencing confirmed a *TP53* mutation, and intriguingly a *TP53* mutant subclone was detected already in the diagnostic sample.[20] As high-risk MDS and AML with del(5q) is tightly linked to *TP53* mutation,[21] a

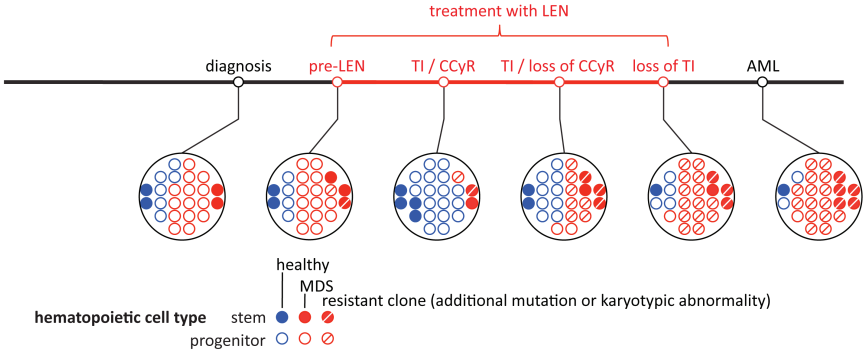

Figure 2. During the clinical course of MDS with del(5q) the proportion of del(5q) marrow cells may be altered by treatment or disease progression. Even when del(5q) is not detected with standard karyotyping (i.e. when the patient is in CCyR), a significant proportion of the hematopoietic stem cells still carry the del(5q). The presence of small subclones harboring adverse genetic events such as *TP53* mutation may substantially predate disease transformation. Early detection of adverse events using highly sensitive methods may have prognostic value. MDS: myelodysplastic syndromes, LEN: lenalidomide, CCyR: complete cytogenetic response, TI: transfusion independency.

likely explanation why *TP53* mutations have not been detected in low-risk MDS with del(5q) is the relative insensitivity of Sanger sequencing, which requires around 30% mutated alleles to be detectable. Deep sequencing of marrow from patients with lower risk MDS and del(5q) (n=55) revealed that 18% had *TP53* mutations, and this was significantly associated with subsequent leukemic evolution.[22] This demonstrates that patients with early stage MDS with del(5q) can harbor adverse marrow subclones that may expand due to acquisition of additional genetic alterations, resulting in disease progression (Figure 2).

Importance of the method of genetic monitoring

There is no consensus about how frequently karyotyping or FISH analysis of 5q should be performed during the routine follow up of patients with del(5q) MDS, nor which method is better for following the del(5q) status. Patients who fail to achieve a cytogenetic response are at high risk of disease progression.[18] Early signs of clonal evolution may motivate treatment modification, such as switching from lenalidomide to 5-azacytidine or evaluation for allogeneic stem cell transplantation.

In this issue of Haematologica, Göhring *et al.* report their experience of genetic monitoring in 302 MDS patients with del(5q) who were treated with lenalidomide in the MDS-003 and MDS-004 trials.[17,23] At diagnosis there was minimal discordance between standard karyotyping and FISH (4%). But surprisingly, at 18 months post-diagnosis, 84 of 267 patients (31%) showed del(5q) by karyotyping, but not by FISH. Furthermore, in 5 of these 84 patients additional cytogenetic changes were found, thus, monitoring with FISH only would have failed to identify the genetic progression. The authors conclude that karyotyping is required to detect clonal evolution and that it is significantly more sensitive than FISH for detecting del(5q). FISH is of particular value when karyotyping is unreliable due to poor chromosome morphology or when less than 25 metaphases can be assessed.

It is not entirely clear why karyotyping is more sensitive than FISH. As the authors indicate, one reason may be that the sensitivity of detecting chromosomal deletions by FISH is limited, with a cut-off of 8%. Culture conditions for karyotyping may also potentially select for the malignant clone or preferentially induce proliferation in the residual del(5q) subpopulation.

Future perspectives

Göhring *et al.* rightly suggest that karyotyping may be critical for monitoring patients receiving lenalidomide, in particular to identify signs of clonal progression that would motivate an altered treatment. Recently, 5q-deletion was identified in primitive hematopoietic cells of del(5q) MDS patients on lenalidomide therapy even when conventional karyotyping was normal.[24] This provides a good explanation as to why the response to lenalidomide is transient, but also raises the question of what the limit of detection should be for monitoring del(5q) patients on lenalidomide, and whether we should be aiming for molecular monitoring of minimal residual disease in this population (Figure 2).

It would be of great value to be able to determine *a priori* which patients will respond to lenalidomide. In this regard, preliminary data suggest that the miRNA or *SPARC* response to *ex vivo* stimulation of $CD34^+$ cells with lenalidomide may be predictive. It will also be crucial to determine whether lenalidomide negatively affects outcome, and to validate the prognostic importance of *TP53* mutations.

Emerging data from next-generation sequencing is likely to dramatically increase our understanding of the genetic basis of disease initiation and progression, enabling improved risk stratification and genetic monitoring. Ultimately, novel drug targets may be discovered. In the near future, this might allow treatment to be tailored based on genetic characteristics and therapy to be modified accordingly if novel changes appear during the course of the disease.

Martin Jädersten acquired his MD PhD at the Karolinska Institutet in Stockholm, and is board certified in internal medicine and hematology. He recently joined Dr. Aly Karsan's lab at the Genome Sciences Centre, British Columbia Cancer Agency (BCCA) for postdoc training. His research interests include the role of treatment with erythroid growth factors and lenalidomide in MDS, as well as the biology of the del(5q) MDS.

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Myelodysplastic syndromes with bone marrow fibrosis

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Structural fibrils constitute a physiological component of the bone marrow stromal microenvironment and contribute to providing a connective tissue structure and a support for hematopoietic progenitor cells.[1] The most common fibers in the bone marrow are reticulin and collagen type I/III. Bone marrow biopsy sections can be examined for stromal fibers using a silver impregnation technique such as Gomori's stain, reticulin fibers having a smaller diameter and a greater content of interfibrillar material compared to collagen.[2]

A wide variety of benign conditions and malignant disorders are associated with a pathological increase in bone marrow stromal fibers.[1] Among hematologic malignancies, several myeloid neoplasms, as defined by the World Health Organization (WHO) classification, are associated with an increase in bone marrow fibrosis, including myeloproliferative disorders (primary myelofibrosis, myelofibrosis secondary to thrombocythemia or polycythemia, BCR-ABL1$^+$ chronic myelogenous leukemia), myelodysplastic/myeloproliferative (MDS/MPN) disorders (chronic myelomonocytic leukemia, refractory anemia with ring sideroblasts and thrombocytosis) and acute leukemia (acute megakaryoblastic leukemia, acute pan-myelosis with myelofibrosis). In addition, bone marrow fibrosis is described in a proportion of patients with myelodysplastic syndromes (MDS).[3]

The pathophysiology of bone marrow fibrosis in these disorders is just beginning to be elucidated. Most diseases with increased bone marrow fibrosis are associated with abnormalities of the number and/or function of megakaryocytes and platelets.[4] Cytokines from megakaryocytes and platelets appear to be necessary, but perhaps not sufficient, for fibrosis to occur. A growing body of evidence suggests that transforming growth factor-β, a potent stimulator of fibroblast collagen synthesis, plays a key role in determining a pathologically increased deposition of bone marrow stromal fibers, but it is likely that other cell types, cytokines and growth factors are also involved.[4]

The clinical implications of increased reticulin seem to be different from those of increased collagen: the amount of bone marrow reticulin shows little correlation with the severity of the underlying hematologic disease while the presence and amount of collagen fibers are strongly correlated with abnormal blood counts and poor outcome. Moreover, reticulin fibrosis is often reversible after therapeutic intervention, while collagen fibrosis is less likely to be modified by treatment.[1]

The use of a clear histological terminology in the definition of bone marrow fibrosis is very important. Until recently the assessment of bone marrow fibrosis was mainly based on subjective evaluations by individual pathologists using different grading systems and methods of processing the trephine biopsies.[1,5] In 2005, a group of European experts (European Myelofibrosis Network, EUMNET) formulated a consensus-based proposal for a semiquantitative evaluation of bone marrow fibrosis with the aims of avoiding excessive overlapping and achieving a high degree of reproducibility in clinical practice.[6] Grading of myelofibrosis was simplified by introducing four categories (including normal reticulin density) and differentiating between reticulin and collagen fibers. Given the high