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Genetic deletions in AML and MDS

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Chromosomal deletions are common molecular events in myeloid malignancies. Heterozygous deletions may contain a tumor suppressor gene that undergoes homozygous inactivation or may contain one or more genes that alter the disease phenotype through haploinsufficiency. The most common karyotypic abnormality in myelodysplastic syndrome (MDS) is deletion of chromosome 5q. A subset of patients with del(5q) as a sole cytogenetic abnormality has a consistent set of clinical features, termed the 5q- syndrome. While no tumor suppressor genes have been identified on 5q that are homozygously inactivated, recent studies have highlighted several genes and micro RNAs (miRNAs) that cause the phenotype of the 5q- syndrome through allelic insufficiency. For example, deletion of one allele of the *RPS14* gene causes a severe defect in erythropoiesis, analogous to the congenital syndrome Diamond Blackfan anemia, which is itself caused by mutations that inactivate one allele of a ribosomal gene. Loss of one allele of miR-145 and miR-146a causes an increase in megakaryocyte production and may contribute to the clonal advantage of cells with del(5q). The functional approaches used to dissect the molecular basis of the 5q deletion in MDS have the potential to identify key genes and therapeutic targets within other chromosomal deletions in hematologic malignancies.

Introduction

In myeloid malignancies, cytogenetic abnormalities have a profound influence on phenotype, prognosis, and response to therapy. Balanced translocations are common in acute myeloid leukemia (AML), the most prevalent lesions being t(8;21), inv(16), and t(15;17) [1]. Patients with each of these translocations have a relatively favorable prognosis, and these lesions are associated with specific

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therapeutic opportunities, including high dose ara-C, all-trans retinoic acid, and arsenic trioxide. Unbalanced chromosomal abnormalities are more common in myelodysplastic syndrome (MDS) and also occur in AML. Trisomy 8 and deletions of part or all of chromosomes 5 and 7 are common events in myeloid malignancies, though recurrent, somatically acquired gains or losses of many other chromosomal segments have been described [2].

Balanced translocations have been more tractable than unbalanced lesions to experimental dissection of molecular mechanisms. The AML translocations are among the best understood genetic abnormalities in human malignancies, while elucidation of the molecular pathophysiology of most common amplifications and deletions has progressed much more slowly. A first step in the characterization of regions with abnormal copy number (gains or losses of chromosomal segments) is the identification of key genes within these loci that contribute to the molecular pathophysiology of the disease. In this regard, substantial progress has been made in the genetic dissection of del(5q), and this deletion has in turn become a model for the study of heterozygous deletions in cancer [3].

5q- Syndrome

While MDS is a heterogeneous disease, patients with the 5q- syndrome have a relatively consistent clinical phenotype, implying that deletion of one or more genes on 5q is responsible for the biological features of this disease subtype. The 5q- syndrome was described in the 1970s and represented the first chromosomal deletion to be associated with a specific hematologic phenotype [4]. MDS patients with isolated deletions of 5q generally have a severe refractory anemia with macrocytosis. Patients are usually transfusion-dependent with a relatively low rate of progression to AML, so iron overload can be an important cause of morbidity and mortality. The platelet count tends to be normal or high, particularly compared to other types of MDS, but the neutrophil count is relatively unaffected or low. Distinctive hypolobated micromegakaryocytes are often apparent on pathologic examination of the bone marrow. For unclear reasons, the 5q- syndrome is more common in women than men [3,5].

Two chromosomal regions have been mapped on chromosome 5q in MDS. One region, at 5q33, is associated with the 5q- syndrome [6]. A second region is located more proximally at 5q31 and has been linked to a more aggressive form of MDS and AML and is often accompanied by additional cytogenetic abnormalities and a poor prognosis [7,8]. While most patients have large deletions that encompass both loci, the rare patients who have deletions of one locus and not the other have been instructive in localizing genes that are critical for the pathophysiology of MDS. Extensive efforts have been undertaken to identify a tumor suppressor on 5q that has both alleles inactivated. Cases of MDS with del(5q) have been analyzed using Sanger and next generation sequencing technologies to identify genes with mutations in the nondeleted allele, by single nucleotide polymorphism (SNP) microarrays to identify microdeletions causing homozygous inactivation of a gene, and by gene expression profiling to identify genes that might be transcriptionally silenced [9–12]. While the *CTNNA1* gene, located at 5q31, is epigenetically silenced in a subset of patients with del(5q), no genes in the 5q33 deletion have been identified with homozygous inactivation. These studies have reinforced the hypothesis that haploinsufficiency for one or more genes on 5q causes MDS.

Ribosomal dysfunction in the 5q- syndrome

Since patients with del(5q) have deletions of many genes, and genetic studies have not been able to localize an individual gene, functional studies are required to identify critical genes. RNA interference is a genetic tool that can be applied to interrogate the function of each gene within a chromosomal deletion. In a screen of the 5q33 common deleted region associated with the 5q- syndrome, short hairpin RNAs (shRNAs) targeting ribosomal protein S14 (*RPS14*) decreased the production of erythroid cells relative to megakaryocytic cells more than any other shRNAs. *RPS14* shRNAs also caused an accumulation of immature cells and cause increased apoptosis of primary human hematopoietic progenitor cells. Overexpression of *RPS14* in cells from MDS patients with the 5q deletion rescued erythropoiesis [13].

Haploinsufficiency for *RPS14* in the 5q- syndrome links this acquired form of MDS to Diamond Blackfan anemia, a congenital disorder in which heterozygous mutations have been identified in ribosomal genes [14]. Patients with Diamond Blackfan anemia, like patients with the 5q- syndrome, have a severe macrocytic anemia. Causal mutations in more than 10 genes have been identified in

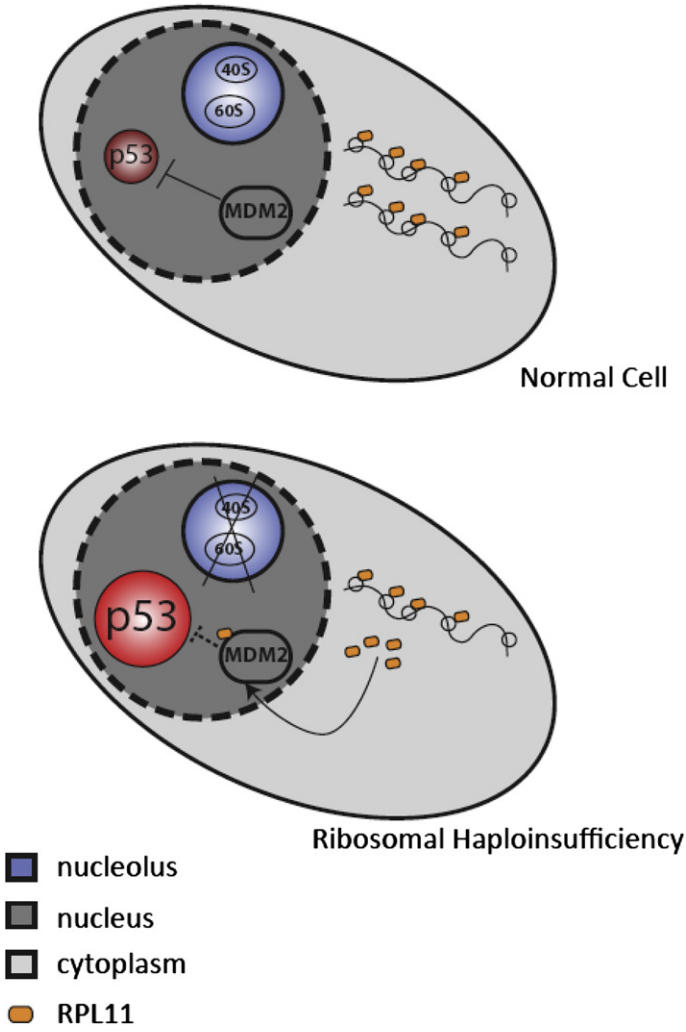

Fig. 1. Schema of a proposed mechanism of p53 activation in response to ribosome dysfunction. Ribosome dysfunction due to haploinsufficiency for a ribosomal protein coding gene leads to ribosome dysfunction, increased levels of RPL11, sequestration of MDM2 by RPL11, and accumulation of p53. This research was originally published in *Blood* [14]. ©The American Society of Hematology.

patients with Diamond Blackfan anemia, all in ribosomal genes (including *RPS19*, *RPS24*, *RPS17*, *RPS7*, *RPS27a*, *RPS15*, *RPS10*, *RPL36*, *RPL14*, and *RPL35A*). To date, ribosomal genes are the only class of genes that have been found to be mutated in this disease, accounting for approximately 50% of cases. The mutations in ribosomal protein genes are heterozygous, resulting in loss of function of one allele. Most likely, inactivation of both alleles of a core ribosomal gene would not be tolerated by a cell, providing one explanation for why deletions of 5q are never homozygous [14].

The p53 pathway plays a central role in the hematopoietic effects of ribosomal gene haploinsufficiency. The p53 pathway not only senses genome instability but also detects aberrant ribosome function. In murine and zebrafish models, ribosomal gene dysfunction causes defective erythropoiesis, and red blood cell production is normalized in the absence of p53 [14]. Notably for the 5q- syndrome, conditional deletion of a 9-gene region, including the *RPS14* gene, causes a macrocytic anemia, and the hematopoietic phenotype is rescued in mice with homozygous knockout of the *p53* gene [15].

A potential mechanism that connects defects in ribosome biogenesis to p53 involves MDM2, an E3 ubiquitin ligase that targets p53 for proteosomal destruction (Fig. 1). Three ribosomal proteins, RPL5, RPL11, and RPL23, bind directly to MDM2, likely inhibiting p53 function. A mechanistic connection between ribosomal protein gene haploinsufficiency and p53 activation was made using mice with conditional knockout of the RPS6 gene. Haploinsufficiency for RPS6 can increase translation of RPL11. RPL11, in turn, binds to MDM2, preventing the inactivation of p53 [16]. The erythroid failure characteristic of the 5q- syndrome and Diamond Blackfan anemia may therefore be due to an MDM2-mediated accumulation of p53, causing cell cycle arrest and apoptosis of erythroid progenitor cells.

Other genes in del(5q) MDS

Functional evidence supports the involvement of multiple genes in the pathogenesis of del(5q) MDS. Two miRNAs have been implicated in the pathogenesis of the 5q- syndrome, acting to increase megakaryopoiesis and to give cells with del(5q) a selective advantage [17,18]. One miRNA, *miR-145*, is located within the 5q33 common deleted region, and the second, *miR-146a*, is located just distal to the locus. Expression of both miRNAs is decreased in MDS patients with del(5q). Decreased expression of these miRNAs increases megakaryopoiesis in murine bone marrow transplantation models and causes the characteristic hypolobated micromegakaryocyte morphology observed in the 5q- syndrome. The gene encoding FLI-1, a megakaryocytic-specific transcription factor, is a target of *miR-145* [17]. Both miR-145 and miR-146a target genes involved in the innate immune system [18].

Murine models have implicated three additional genes in the pathophysiology of del(5q) MDS [19–23]: *EGR1*, *APC*, and *NPM1*. *EGR1* is located within the 5q31 common deleted region, *APC* is located just proximal to this locus, and *NPM1* is located at 5q35. Heterozygous inactivation of *EGR1* increases hematopoietic stem cell self-renewal. Heterozygous inactivation of *APC* increases beta catenin activity, leading to a myeloproliferative phenotype in mice. Mice with heterozygous inactivation of *NPM1* develop dysplastic erythropoiesis and genomic instability. The gene encoding CTNNA1, located within the 5q31 deletion, is hypermethylated in a subset of cases with MDS.

Another striking aspect of the clinical phenotype of the 5q- MDS patients is their tremendous response to the immunomodulatory agent lenalidomide. Haploinsufficiency for two phosphatases on 5q, *PP2A* and *CDC25C*, have been implicated in the sensitivity of cells with del(5q) to lenalidomide [24].

In aggregate, these studies provide convincing evidence that the 5q deletion in MDS causes haploinsufficiency for multiple genes, and the full phenotype of the disease is caused by decreased gene dosage for multiple contiguous genes. The vast majority of patients has deletions that encompass both common deleted regions, including 5q31 and 5q33, and therefore have heterozygous deletion of *RPS14*, *miR-145*, *miR-146a*, *EGR1*, *CTNNA1*, and *APC*. A smaller proportion has deletions that include *NPM1*.

Conclusion

Heterozygous deletions in cancer can result in haploinsufficiency for multiple genes, even in the absence of homozygous inactivation of any genes. The biological consequences of the deletion may therefore result from the integrated effects of decreased expression of multiple genes. Similarly, the phenotypic consequences of amplifications of whole chromosomes, such as trisomy 8, may also be due to increased gene dosage for multiple genes. In the absence of genetic evidence highlighting a single gene, functional studies are required to dissect the genetics of large heterozygous deletions.

Functional studies of the 5q deletion in MDS have highlighted the *RPS14* gene, as well as *miR-145* and *miR-146a*, in the pathophysiology of the 5q- syndrome. Haploinsufficiency for *RPS14* in del(5q) links the 5q- syndrome to Diamond Blackfan anemia. Both diseases are characterized by haploinsufficiency for a ribosomal protein gene, and both cause a severe macrocytic anemia that is likely mediated by p53 activation. The poor prognosis associated with patients with 5q deletions and multiple cytogenetic abnormalities may be attributable to the genetic or epigenetic inactivation of p53. The approaches used to study the 5q deletion in MDS are now being applied to other chromosomal deletions in myeloid malignancies and will hopefully lead to novel insights into molecular basis of AML and MDS.

Conflict of interest statement

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