

The role of haploinsufficiency of RPS14 and p53 activation in the molecular pathogenesis of the 5q- syndrome

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Abstract

In recent years we have gained great insight into the molecular pathogenesis of the 5q- syndrome, a distinct subtype of myelodysplasia. The demonstration of haploinsufficiency of the ribosomal gene *RPS14* (mapping to the commonly deleted region) and the finding that this is the cause of the erythroid defect in the 5q- syndrome represent major advances. A mouse model of the human 5q- syndrome generated by large-scale deletion of the *Cd74-Nid67* interval (containing *RPS14*) further supports a critical role for *RPS14* haploinsufficiency. It is widely accepted that ribosomal deficiency results in p53 activation and defective erythropoiesis and the crossing of the '5q- mice' with p53 deficient mice ameliorated the erythroid progenitor defect. Emerging data suggests that the p53 activation observed in the mouse model may also apply to the human 5q- syndrome.

The 5q- syndrome

The 5q- syndrome was first described in 1974 by Van den Berghe who reported the consistent association of the deletion of the long arm of chromosome 5 [del(5q)] with the following hematological features: macrocytosis, anemia, normal or high platelet count and hypolobulated megakaryocytes in the bone marrow.¹ From the earliest studies a good prognosis and a marked female preponderance have also been reported.^{2,3} The 5q- syndrome is widely considered the most distinct of all the myelodysplastic syndromes and, importantly, this MDS subtype is characterized by a clear genotype-phenotype relationship which is not observed in other MDS and acute myeloid leukemia (AML) characterized by chromosomal deletions. The 5q- syndrome is now recognized as a distinct clinical entity according to the WHO classification and is defined by a medullary blast count of <5% and the presence of the del(5q) as the sole karyotypic abnormality.⁴

Mapping the commonly deleted region and identifying candidate genes

The identification of significant genes associated with chromosome deletions in human leukaemia has proven challenging. The del(5q) in the 5q- syndrome is considered to mark the location for a gene(s) the loss of which may affect important processes such as growth control and normal hematopoiesis.² The basis for research on deletions such as the del(5q) in the 5q- syndrome is well known. The first step is to characterize the deletions and to identify the commonly deleted region (CDR) i.e. the region of deletion shared by all patients as this localises the gene(s) for further study. Our group in Oxford identified the CDR of the 5q- syndrome^{5,6} and have since narrowed the CDR to the approximately 1.5 Mb interval at 5q32 flanked by D5S413 and the *GLRA1* gene.⁷ We subsequently generated a transcription map of the CDR and noted several promising candidate genes map within this region, including the tumour suppressor gene *SPARC*, and *RPS14*, a component of the 40S ribosomal subunit.^{7,8} The next step in our research was the sequencing of all the 40 genes that map within the CDR in a group of patients with the 5q- syndrome.⁸ The gene sequencing is critical to understanding the pathogenesis of the 5q- syndrome; if Knudsen's two hit model⁹ applied to this disorder there would be loss of one allele of a gene and a mutation of the remaining copy of the same gene. We have sequenced all the genes in the CDR and no mutations have been identified.⁸ This is a key step in determining the molecular basis of the 5q- syndrome since it brings forward the consideration of haploinsufficiency (a gene dosage effect resulting from the loss of one allele of a gene)¹⁰ as the basis of the 5q- syndrome. There has been growing recognition of haploinsufficiency as a cancer model over the last decade and we believe that this is the correct model for the 5q- syndrome.

Haploinsufficiency of RPS14 causes the erythroid defect in the 5q- syndrome

We have recently demonstrated haploinsufficiency of the ribosomal gene *RPS14* in the CD34+ cells of patients with the 5q- syndrome.⁸ The genes in the 5q- syndrome CDR were studied by an RNA-mediated interference (RNAi)- based approach by Ebert *et al.* and it was shown that partial loss of function (haploinsufficiency) of *RPS14* in normal hematopoietic stem cells resulted in a block in erythroid differentiation with relative preservation of megakaryocyte differentiation¹¹ closely mirroring the defects observed in the 5q- syndrome. Moreover, forced expression of

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an *RPS14* cDNA in primary bone marrow cells from patients with the 5q- syndrome rescued the phenotype, strongly suggesting that *RPS14* is a 5q- syndrome gene.¹¹

That reduced expression of a ribosomal protein plays a key role in the development of a myeloid malignancy may initially seem surprising. However, Diamond-Blackfan anaemia (DBA), a congenital hypoplastic anemia, is also caused by haploinsufficiency of certain ribosomal proteins. The phenotype of DBA is remarkably similar to the 5q- syndrome since patients have a severe anaemia, macrocytosis, relative preservation of the platelet and neutrophil counts, erythroid hypoplasia in the bone marrow and an increased risk of leukaemia.¹² Mutations in *RPS19*, required for the maturation of 40S ribosomal subunits, are found in approximately 25% of DBA patients and lead to haploinsufficiency of *RPS19*.^{13,14} Deficiency of *RPS19* has been shown to block proliferation of immature erythroid progenitor cells.¹⁵ Interestingly DBA has been associated with mutations now in a number of different ribosomal protein genes including *RPS19*, *RPS24*, *RPS17*, *RPL35A*, *RPL5*, *RPL11*, and *RPS7* in approximately 43% of patients.¹⁶ Importantly, an acquired internal deletion on chromosome 5q involving the CDR of the 5q- syndrome (and loss of *RPS14*) has been recently described in a patient with non-classical DBA.¹⁷

The anaemia in DBA and the 5q- syndrome is due to a failure of erythropoiesis and intriguingly both disorders show haploinsufficiency for ribosomal proteins, *RPS19* and *RPS14* respectively, required for the maturation of 40S ribosomal subunits.^{13,18} Mutations in *RPS19* impair pre-rRNA processing of the 18S rRNA, which leads to decreased production of the 40S ribosomal subunit¹⁹ and *RPS14* haploinsufficiency causes a similar outcome,¹¹ thus linking the pathogenesis of the 5q- syndrome to

DBA. Moreover, similarities in the defective gene expression patterns observed in the CD34+ cells of patients with DBA and patients with the 5q- syndrome were recently reported by Pellagatti *et al.*, including the down-regulation of multiple ribosomal genes and genes involved in translation initiation and the up-regulation of several proapoptotic genes, further suggesting that the 5q- syndrome represents a disorder of aberrant ribosome biogenesis.²⁰ These abnormalities may lead to impairment of ribosome biogenesis and subsequent reduction of protein translation capacity, a defect which may be of particular importance for developing erythroid cells, whose survival and division require large amounts of protein synthesis.

A number of other bone marrow failure syndromes are caused by defects in genes known to play a role in ribosome biogenesis including Shwachman-Diamond Syndrome,²¹ Dyskeratosis Congenita, Cartilage Hair Hypoplasia, and the demonstration of *RPS14* as a 5q- syndrome gene further suggests that defective ribosomal biogenesis may have a more general relevance in leukaemogenesis.²²

P53 activation underlies the anemia in the 5q- syndrome

P53, a key regulator of cell growth and cell death, is maintained at a low level during normal cell growth and is activated in response to various cellular stresses. Impaired ribosomal biogenesis, such as that resulting from haploinsufficiency of certain ribosomal proteins can cause nucleolar stress.^{23,24} Signal mediator proteins then activate p53 by inhibiting ubiquitylation (and degradation) by MDM2, the major regulator of p53.²⁵ Activated p53 then promotes the transcription of its many target genes resulting in p53-dependent cell cycle arrest or apoptosis. It is now widely recognised that p53 activation is a common response to deficiency of ribosomal proteins in various diseases including DBA and Treacher Collins syndrome.^{24,26}

We have recently generated a mouse model of the 5q- syndrome using large-scale chromosomal engineering.²⁷ Haploinsufficiency of the *Cd74-Nid67* interval caused macrocytic anemia, prominent erythroid dysplasia and monolobulated megakaryocytes in the bone marrow. The *Cd74-Nid67* interval on mouse chromosome 18 is syntenic with a region within the CDR of the human 5q- syndrome⁷ and contains 8 known genes of which 2 have been excluded (*Ndst1* and *Cd74*) leaving *Rps14*, *Synpo*, *Myoz3*, *Dctn4*, *Rbm22* and *Nid67* as candidates.²⁷ It is most probable that *RPS14* is the major gene in relation to the phenotype. The '5q- mouse' has a defective bone marrow progenitor development and bone marrow cells expressing high amounts of p53 with increased apoptosis. The 5q- mouse was crossed with p53 deficient mice and significantly this rescued the progenitor

cell defect, restoring hematopoietic stem cell bone marrow populations.²⁷ These data suggest that a p53-dependent mechanism underlies the pathophysiology of the 5q- syndrome.

We have recently shown that induction of p53 and up-regulation of the p53 pathway occurs in the human 5q- syndrome²⁸ and Dutt *et al.* have demonstrated that haploinsufficiency of *RPS14* indeed causes activation of p53 in human erythroid cells.²⁹

Cooperating events in the development of the 5q- syndrome

There is compelling data suggesting that haploinsufficiency of *RPS14* gene causes the erythroid differentiation defect of the 5q- syndrome^{11,27} and there is evidence suggesting that loss of the miRNA genes miR-145 and miR-146a (mapping within and adjacent to the CDR of the 5q- syndrome, respectively) may play a role in the abnormalities of the megakaryocyte lineage observed.³⁰ Thus several cooperating genetic events may be necessary in the development of the 5q- syndrome.³¹ Whether *RPS14* or these microRNA genes are causal genes in relation to producing a clonal haematopoietic disorder, and whether haploinsufficiency of an additional gene or genes is involved are important questions remaining to be answered. Haploinsufficiency of other genes localised within the CDR such as the tumour suppressor gene *SPARC*, or indeed mutations in genes mapping elsewhere in the genome, could play a role in establishing clonal dominance.³² Clearly further mouse knockout models and whole genome sequencing studies might prove very informative in relation to all these questions.

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