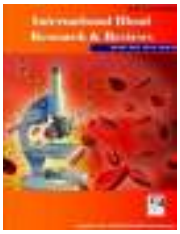

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Lenalidomide, p53 and del(5q) Myelodysplastic Syndrome: Ribosome Stress Relief

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DGY and JML designed and wrote the manuscript. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Whereas deletions involving the long arm of chromosome 5 are among the most common chromosomal abnormalities in myelodysplastic syndrome (MDS), isolated del(5q) MDS, which includes the 5q- syndrome, is rare and characterized by hypoplastic anemia and a moderate risk of transformation to acute myeloid leukemia (AML). The 5q- syndrome is now recognized as a ribosomopathy, and both the classic 5q- syndrome and del(5q) MDS are uniquely responsive to lenalidomide. However, the mechanism of action of lenalidomide is controversial and involves modulation of p53 activity, which may be beneficial in anemia remission but suggested to lead to malignant cell outgrowth. Here, we critically review the literature on this important controversy, which has obvious implications for therapy of del (5q) MDS.

Keywords: Lenalidomide; p53; ribosome; del(5q) MDS; Diamond Blackfan anemia.

1. 5q- SYNDROME IS A RIBOSOMOPATHY

Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoiesis and progression to acute myeloid leukemia (AML). Deletions involving the long arm of chromosome 5 are among the most common chromosomal abnormalities in MDS (being

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present in approximately 20-30% of patients), whereas isolated deletion of 5q is relatively uncommon and is often referred to as the 5q- syndrome, which is usually characterized by macrocytic anemia, erythroid hypoplasia, thrombocytosis, and megakaryocyte dysplasia [1]. However, not all patients with isolated del(5q) MDS have all the classical features of the 5q- syndrome [2], and "MDS with isolated del(5q)" is defined by the World Health Organization classification by a medullary blast count of less than 5% (less than 1% blasts in the peripheral blood) and presence of the del(5q) as the sole karyotype anomaly, without specifying megakaryocyte morphology or other clinical features. The major cause of morbidity and mortality is the erythroid defect, which often requires recurring erythrocyte transfusion.

In 2008, Ebert and colleagues demonstrated that the refractory anemia associated with the 5q- syndrome is caused by haploinsufficiency (a gene dosage effect resulting from the loss of one allele of a gene) for the ribosomal protein (RP) S14 [3]. This revelation tied the 5q- syndrome to the genetic pure red cell aplasia, Diamond Blackfan anemia (DBA), which is also largely due to haploinsufficiency of RPs, with both syndromes classified as so-called ribosomopathies (disorders of ribosome biogenesis). Presumably, these disorders may lead to reduction of protein translation capacity, which might be particularly deleterious to developing erythroid cells, whose survival and division require large amounts of protein synthesis. Alternatively, these ribosomopathies may lead to specific translational defects in the erythron. Moreover, multiple animal models have linked the effects of ribosomal dysfunction on erythropoiesis with the p53 tumor suppressor, which is known to monitor ribosome integrity [4]. Consequently, allelic deficiency of RPS14 may contribute to a unique pathophysiology closely related to p53.

2. *TP53* IN 5q- SYNDROME AND Del(5q) MDS

In the p53 signaling pathway, the p53 tumor suppressor protein plays a central role in coordinating a complex network that prevents aberrant cell growth and proliferation. In normal conditions, p53 is maintained at low steady state levels. Crucial for this regulation are two proteins, murine double minute Mdm2 and Mdmx (HDM2 and HDMX in humans). Deregulated functions of these p53 inhibitory proteins are critical for an activated p53 response in stress situations. Overexpression of oncogenes stimulates the production of alternative reading frame, ARF, which binds to Mdm2 and stabilizes p53. Activated p53 subsequently trans-activates many downstream target genes involved in apoptosis or cell cycle arrest.

Impaired ribosomal biogenesis, such as that resulting from haploinsufficiency of certain ribosomal proteins, can cause nucleolar stress. In response to this stress, some ribosomal proteins that are liberated as a result of abortive assembly bind to Mdm2 and block Mdm2-mediated p53 ubiquitination and degradation, resulting in p53-dependent cell inhibition[5]. Hence, it is widely accepted that p53 activation is a common response to deficiency of ribosomal proteins in the so-called ribosomopathies (including Diamond Blackfan anemia and the 5q- syndrome). Elevated levels of p53 and increased bone marrow cell apoptosis were observed in a mouse model of 5q- syndrome that displays macrocytic anemia and erythroid dysplasia[6]. Notably, when the 5q- deletion is introduced into a p53- null background, the hematopoietic phenotypes of the mutant mice can be completely rescued. Boultwood et al's 5q- mouse model exhibits a defective bone marrow progenitor development, with bone marrow cells expressing high amounts of p53 associated with increased apoptosis [7].

Subsequently, Dutt et al examined whether p53 accumulates differently among lineages in response to decreased expression of ribosomal proteins[8]. Their work suggested that activation of the p53 pathway resulted in erythroid-specific cell cycle arrest and apoptosis, consistent with the hematopoietic phenotypes of the 5q- syndrome and DBA. They also found an accumulation of p53 in erythroid progenitor cells in bone marrow biopsies from patients with del(5q) MDS and DBA, which corresponds with their in vitro results.

The p53 pathway may play a particularly important role in the erythroid lineage, improving the efficiency of erythropoiesis and preventing malignant transformation. For example, induction of p53 in erythroid progenitor cells with genetic and ribosomal abnormalities could prevent the needless expenditure of energy to produce dysfunctional erythrocytes. In addition, sensitivity of erythroid progenitor cells to p53 activation could prevent the development of leukemia in response to genomic stress.

3. WHAT ABOUT THROMBOCYTOSIS IN THE 5q- SYNDROME?

Thrombocytosis and megakaryocyte hyperplasia with nuclear hypolobation are classic associated features of the 5q- syndrome. Although haploinsufficiency of RPS14 recapitulates the defect in erythroid differentiation in 5q- syndrome, it does not appear to influence thrombocytosis. Rather, deletion of the microRNA (miR) genes miR-145 and miR-146a, mapping within and adjacent to the common deleted region (CDR) in the 5q- syndrome, has been suggested to contribute to the thrombocytosis and megakaryocytic dysplasia [9-11].

Recent publications [10,12] have further defined the function of miR-145 as repressing Fli-1, an ETS-family transcription factor originally found as an insertion site of the Friend leukemia virus, which plays a role in both megakaryocytic and erythroid differentiation. Overexpression of Fli-1 in leukemia cells induces megakaryocyte differentiation while suppressing erythroid differentiation. Thus, del(5q) MDS patients were found to have decreased expression of miR-145 and increased expression of Fli-1 [10]. The combined loss of miR-145 and RPS14 was postulated to alter erythroid-megakaryocytic differentiation to contribute to the full phenotype of the 5q- syndrome.

In mice, Fli-1 apparently regulates p53 by activating the transcription of Mdm2 [13]. Consequently, elevated Fli-1 expression in the 5q- syndrome may protect megakaryocytic cells from p53-ribosomal stress, thereby contributing to thrombocytosis and dysplastic megakaryopoiesis [12].

4. LENALIDOMIDE And p53 MODULATION: GOOD or BAD?

Recently the thalidomide analog, lenalidomide, has been shown to induce durable erythroid responses in patients with MDS. Specifically in patients with 5q- syndrome and del(5q)-associated MDS, transfusion independence and cytogenetic remissions can be observed. However, up to 50% of these patients may experience clinical and cytogenetic relapse after 2-3 years of treatment, raising the possibility of continued clonal evolution, which is then resistant to lenalidomide [14].

How lenalidomide functions in del(5q) MDS has been the subject of controversy [15,16]. Lenalidomide has pronounced therapeutic effects in MDS patients with del(5q), exerting several anti-angiogenic and anti-inflammatory actions on cytokine production and modulation of T-, NKT-, T-regulatory and NK-cell functions[reviewed in [16]. Although lenalidomide

seems to primarily target the bone marrow microenvironment, the drug has been described to have a direct effect on clonal MDS hematopoietic cells through inhibition of proteins critical for cell survival or stimulation of tumor suppressor genes in the 5q region [17,18]. These studies suggested that the primary action of lenalidomide is cytotoxicity against del(5q) MDS clones[19]. However, this mechanism of action has recently been challenged[20].

In del (5q) MDS, leukemic evolution occurs in around 12% of patients during the natural course of the disease [21], while the risk of progression is higher in the presence of additional cytogenetic abnormalities or an increased number of blasts. In the lenalidomide era, however, the cytogenetic characteristics at the time of transformation have not been well studied. A preliminary report indicated that patients with del(5q) MDS who did not achieve erythroid or cytogenetic remission after treatment with lenalidomide were at high risk for clonal evolution and acute myeloid leukemia [22]. Some of the 42 patients from this cohort had isolated del(5q), but many had del(5q) with at least one additional chromosomal abnormality. Subsequently, Tehranchi et al described persistent malignant stem cells in a small cohort of classical 5q- syndrome patients who had achieved erythroid and cytogenetic remission after treatment with lenalidomide [14]. As a result of these concerns, the European Medicines Agency (EMEA) initially advised against the approval of lenalidomide in Europe for patients with low-risk MDS and del(5q) stating that a treatment-associated increase in the risk of leukemic transformation could not be excluded.

The RP-Mdm2-p53 pathway is a critical effector of the hypoplastic anemia in patients with del(5q) MDS and congenital anemias arising from RP gene mutations. Both pharmacological inhibition of p53 activity in del(5q) MDS progenitors and genetic p53 inactivation in the syngeneic murine model of the human 5q- syndrome are sufficient to rescue the hematological phenotype, emphasizing the key role of p53 in the molecular pathogenesis of the syndrome. One study found that p53 is overexpressed in a lineage-restricted manner in erythroid precursors of primary human bone marrow del(5q) MDS specimens, and treatment with lenalidomide restores Mdm2 stability to promote p53 degradation in both a cell line model and primary del(5q) MDS specimens, accompanied by suppression of downstream p53 effector genes[20]. This surprising finding raises the possibility that lenalidomide's mechanism of action might involve relieving ribosome stress (and stress-induced p53-mediated growth inhibition), which might be good in the short term but bad in the long term.

Tehranchi et al identified rare and phenotypically distinct del(5q) MDS stem cells that were selectively resistant to therapeutic targeting with lenalidomide at the time of complete and clinical and cytogenetic remission in seven patients with 5q- syndrome [14]. Another study using a sensitive deep sequencing technique identified small clones harboring mutations in the DNA-binding domain of p53 in a subset of patients with del(5q) MDS, which was associated with an increased risk of disease progression and expansion of the mutant clone [23,24]. These findings support the hypothesis that lenalidomide may destabilize p53 and raise questions as to whether checkpoint abrogation with lenalidomide treatment might modify the potential for expansion of p53-mutant clones or modulate DNA repair (or telomere length) in patients receiving concomitant treatment with DNA-damaging agents [20,25,26].

5. WHAT'S THE TARGET OF LENALIDOMIDE?

The E3 ubiquitin ligase protein, cereblon (CRBN) has been identified as a direct molecular target for the teratogenicity of thalidomide and the anti-proliferative activity of lenalidomide [27,28]. CRBN is highly conserved and ubiquitously expressed in human cells. CRBN along

with the DNA damage binding protein-1 (DDB1), Cullin 4A and Roc1 constitute a functional E3 ubiquitin ligase complex, which acts to ubiquitinate target proteins for proteasomeal degradation [27]. CRBN itself undergoes auto-ubiquitination, and thalidomide is described as an inhibitory molecule for CRBN auto-ubiquitination.

In studies on the action of lenalidomide in multiple myeloma, CRBN was found to be required for the anti-myeloma activity of thalidomide and lenalidomide [29]. Low CRBN expression was found to correlate with resistance to lenalidomide in myeloma cells. Although not published yet, it is conceivable that CRBN may be the critical drug target in MDS that is responsive to lenalidomide.

Two target genes [18] are commonly co-deleted in del(5q) MDS but are not included in the critical deleted region (CDR). Both encode phosphatases: *CDC25c* (cell division cycle 25c) and *pp2Acα* (protein phosphatase 2Ac α subunit). The *CDC25c* gene is translated into a protein called M-phase inducer phosphatase, which dephosphorylates a complex of cyclin B and cyclin-dependent kinase 1 and triggers entry into mitosis (M phase) [30]. The *pp2Acα* gene encodes another phosphatase that is critical in the activation of CDC25c [31]. Experiments that knocked down expression of these two genes in cell line models or in bone marrow cells from non-del(5q) patients rendered the cells susceptible to lenalidomide cell cycle arrest and apoptosis [18]. Consequently, haploinsufficiency for the two phosphatases, which is often present due to co-deletion with the del(5q), was proposed as conferring sensitivity of cells to lenalidomide. Although this is an elegant explanation of lenalidomide's activity in del(5q) MDS, our group has recently identified two unusual cases of 5q- syndrome in patients who presented in childhood. In these two individuals, both alleles of the phosphatase genes were retained, and one responded to lenalidomide (Vlachos et al, manuscript submitted, 2013). Our new observations not only cast doubt on the absolute requirements for lenalidomide response, but they also beg broader questions about the authentic target of lenalidomide. Could lenalidomide specifically target cells that are haploinsufficient for RPS14 alone? Furthermore, are there lenalidomide-responsive patients who harbor occult ribosomal protein gene mutations?

Patients with International Prognostic Scoring System (IPSS) low and intermediate-1 risk MDS patients without the del(5q) karyotype have been described to respond to lenalidomide [32]. However, the maximum increase in hemoglobin, the duration of response, and the degree of neutropenia and thrombocytopenia are all less than is typical in del(5q) MDS. This has led to the suggestion that lenalidomide acts on different targets in del(5q) MDS versus non-del(5q) MDS [15]. However, the currently available data on responsiveness to lenalidomide has usually only included analysis of patients identified as del(5q) by interphase karyotyping. It remains possible that MDS responders to lenalidomide might have microdeletions of *RPS14*, for example, which are undetectable by standard cytogenetic methods. In that case, the true target of lenalidomide might only be found by comparison of the expression of specific genes, by molecular methods, in responders and non-responders.

6. CONCLUSION

In conclusion, a final determination of how lenalidomide works in 5q- syndrome and del(5q) MDS has not yet been reached despite a plethora of experiments. If lenalidomide does indeed modify the potential for expansion of p53 mutant clones, it would seem prudent to identify and follow patients' *TP53* mutation status in order to assess prognosis as well as select and/or monitor therapy [25,33].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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