Deregulation of innate immune signaling in myelodysplastic syndromes is associated with deletion of chromosome arm 5q

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Myelodysplastic syndromes (MDS) are a family of heterogeneous clonal hematological malignancies defined by peripheral cytopenias, a hypercellular or normal
marrow with ineffective hematopoiesis, and a propensity to progress to acute myeloid leukemia (AML)\(^1\). The complexity and heterogeneity of MDS, insufficient description of disrupted genetic networks, and lack of mouse models has stymied our understanding and treatment of this disease. Recent seminal findings provide insight into a subtype of MDS called 5q- syndrome, which is associated with an isolated deletion of chromosome arm 5q. 5q- syndrome originates in a hematopoietic stem cell and is defined by an isolated heterozygous deletion of chromosome arm 5q, macrocytic anemia, variable neutropenia, and abnormal megakaryocytes associated with elevated platelets\(^2\). Exploration of candidate protein-coding genes within the minimally deleted region on chromosome 5q revealed a critical role of RPS14 in erythroid apoptosis and macrocytic anemia in mice\(^3\). Our group investigated the contribution of small non-coding RNAs, referred to as microRNAs (miRNAs), to the pathogenesis of 5q- syndrome\(^6\). We have recently reported that deletion of chromosome arm 5q in MDS patients results in loss of at least 2 functionally-related miRNAs, miR-145 and miR-146a. We identified Mal/TIRAP as a target of miR-145, and confirmed TRAF6 as a target of miR-146a. TIRAP and TRAF6 are two proteins of the innate immune pathway, therefore, loss of either or both of miR-145 and miR-146a would be expected to derepress innate immune signaling. By generating mouse bone marrow chimeras in which miR-145 and miR-146a are reduced or TRAF6 is overexpressed, we observed characteristic megakaryocytic dysplasia, elevated platelets, and clonal dominance associated with either bone marrow failure or acute myeloid leukemia (Figure 1). Although loss of miR-145 or miR-146a and TRAF6 overexpression in primary mouse hematopoietic cells resulted in enhanced survival by a cell intrinsic mechanism, increased megakaryocytes and platelets result in part because of
paracrine expression of interleukin-6 (IL-6). Elevated IL-6 expression was observed in TRAF6-activated cells, and marrow of patients with deletion of chromosome 5q showed increased levels of IL-6 mRNA. Macr

ocytic anemia was not observed following loss of miR-145 and miR-146a, suggesting that distinct mechanisms regulate erythroid and megakaryocytic defects in 5q- syndrome. In support of this hypothesis, it is reported that coordinate loss of miR-145 and RPS14 cooperate to alter both erythroid and megakaryocytic production similar to patients with 5q- syndrome. In these mice, erythroid hypoplasia was due to loss of RPS14, while loss of miR-145 increased megakaryopoiesis in transgenic mice. In this latter study, megakaryocyte production was attributed to increased expression of FLI1 (Friend leukemia virus integration 1), a target of miR-145. Interestingly, IL-6 is a potent inducer of FLI1 mRNA expression in human leukemic cells. It is tempting to speculate that FLI1 mRNA and protein are simultaneously increased in 5q- syndrome through activation of TRAF6/IL-6 and loss of miR-145, respectively (Figure 1).

Consistent with our findings, aberrant innate immune signaling due to chronic infection can reproduce hematological abnormalities resembling MDS and result in misdiagnosis. The innate immune system is an evolutionarily conserved defense mechanism against foreign pathogens. The Toll-like receptor (TLR) family is involved in the initial detection and subsequent elimination of foreign pathogens through mechanisms leading to activation of NF-κB and MAP kinases. Given the prominent role of the innate immune pathway in regulating hematopoiesis following infection, it is not surprising that aberrant signaling of this pathway is associated with MDS and AML. The relevant downstream signaling pathways activated following loss of miR-145 and miR-146a in
MDS are not known, but NF-κB activation in a TRAF6- and TIRAP-dependent manner was observed⁹. In line with our observation, there is increased NF-κB activation in CD34⁺ bone marrow cells isolated from low-risk and high-risk MDS patients and cell lines⁹. NF-κB inhibition results in cell death of MDS and normal CD34⁺ cells in vitro, suggesting a role of NF-κB in cell survival and clonal dominance. The contribution of NF-κB to dysplastic bone marrow cells has not been reported, however constitutive activation of NF-κB in mice lacking IκBα results in dysplastic granulocytes and megakaryocytes in the bone marrow¹⁰.

Our finding that the innate immune pathway is activated following loss of two microRNAs on chromosome 5q is the first to experimentally validate a role of this pathway in MDS. Given the heterogeneity and complexity of MDS, it is likely that deregulation of multiple signaling pathways contribute to this hematological disease.

**Figure Legend**

**Figure 1. Schematic of molecular alterations associated with 5q- syndrome.** Deletion of chromosome 5q results in loss of genes, including RPS14, miR-145, and miR-146a. The former two genes are within the minimally deleted region, while miR-146a is telomeric to this region but deleted in approximately 75% of patients. Loss of RPS14 in hematopoietic cells is associated with erythroid defects and macrocytic anemia. Loss of miR-145 is associated with derepression of FLI1 and TIRAP. Loss of miR-146a is associated with derepression of TRAF6. Coordinated activation of TIRAP and TRAF6 (two genes of the innate immune pathway) mediate increased survival of hematopoietic cells resulting in clonal dominance, while expression of IL-6 results in paracrine-mediated stimulation of megakaryopoiesis and platelet production. FLI1 also contributes to enhanced megakaryopoiesis and platelet production. IL-6 is known to increase transcription of FLI1 mRNA levels in hematopoietic cells, thus further augmenting FLI1 expression.
References