Chapter 3
Biology and Pathophysiology of MDS with del(5q)

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Introduction

Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal hematopoietic stem cell malignancies characterized by morphological dysplasia and ineffective hematopoiesis. Clinical manifestations depend upon the lineage(s) affected, with anemia the most common accompanied by red blood cell transfusion dependence in approximately one-third of patients at diagnosis [1]. Understanding of the clinical outcomes associated with specific chromosomal aberrations in MDS has led to the development of prognostic models, including the International Prognostic Scoring System (IPSS) and later revised-IPSS (R-IPSS) [2, 3]. Cytogenetic aberrations are frequently observed in de novo MDS patients with some studies reporting them in >50% of patients [4, 5]. The most common aberration is an interstitial deletion of chromosome 5q (del(5q)) occurring in approximately 15% of patients with most having an isolated del(5q) [4]. As molecular testing such as next-generation sequencing (NGS) became more widely available, several recurring somatic gene mutations were identified in MDS that carry prognostic significance [6]. In particular, *TP53* gene mutations confer the worst overall survival, associated with a significantly greater risk of acute myeloid leukemia (AML) transformation [6, 7]. *TP53* mutations occur in 20% of patients with isolated del(5q) and in 70–100% of patients with complex karyotype including del(5q) supporting a strong correlation with *TP53* mutations and del(5q) [8].

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MDS with isolated del(5q) represents a distinct pathologic subtype defined by the 2016 World Health Organization (WHO) as the constellation of dysplasia in 1 to 3 lineages, bone marrow blasts <5%, circulating peripheral blasts <1%, and the presence of del(5q) alone or with 1 additional chromosomal abnormality with the exception of chromosome 7 deletion or del(7q) [1]. Some of the unique features associated with del(5q) were first recognized in 1974 by Van den Berghe et al. [9]. It was initially referred to as the "5q- syndrome" and was described in a series of patients with macrocytic anemia, dyserythropoiesis with erythroid hypoplasia, a normal or elevated platelet count, and hypolobulated megakaryocytes with an indolent natural history. Our understanding of the biology and pathophysiology of del(5q) MDS has been transformed in recent years with the sequencing of the human genome (Fig. 3.1). Herein, we will describe each major milestone and the remarkable story of discovering how haploinsufficiency accounts for the hematological phenotype and selective sensitivity to the drug lenalidomide in del(5q) MDS.

Pathobiology of del(5q) MDS

In the mid-1990s, genetic mapping techniques were utilized for molecular delineation of the commonly deleted region (CDR) of chromosome 5q in myeloid malignancies, encoded within a 1–1.5 Mb segment [10]. Further investigations in patients with 5q- syndrome localized the distal CDR to 5q32-33 containing 40 genes, of which 33 were transcriptionally haplodeficient in CD34+ cells, representing candidate genes possibly contributing to the disease [11, 12]. A second, more proximal CDR was identified at 5q31 and thought to contain tumor suppressor gene(s) in patients predominantly with therapy-related MDS (excluding cases of 5q- syndrome) and AML [13]. However, subsequent studies indicate most patients have large interstitial deletions that span both CDRs [14]. Notably, only rare somatic point mutations were identified in the remaining alleles, suggesting that the hematological phenotype is dictated simply through genetic haploinsufficiency [15–17]. A number of genes in both the distal and proximal CDRs have since been implicated in the pathogenesis of del(5q) MDS through haploinsufficiency, and they are summarized in Fig. 3.2 [18].

Haploinsufficiency Underlies Hematologic Phenotype

In seminal investigations by Ebert and colleagues using short hairpin RNAs (shRNA) to knockdown each of the distal CDR candidate genes in normal CD34+ human hematopoietic progenitor cells identified the ribosomal processing S14 (*RPS14*) gene as the key determinant of the hypoplastic anemia in del(5q) MDS [19]. The *RPS14* gene encodes a key component of the 40S ribosomal subunit, which when underexpressed results in a severe decrease in production and survival

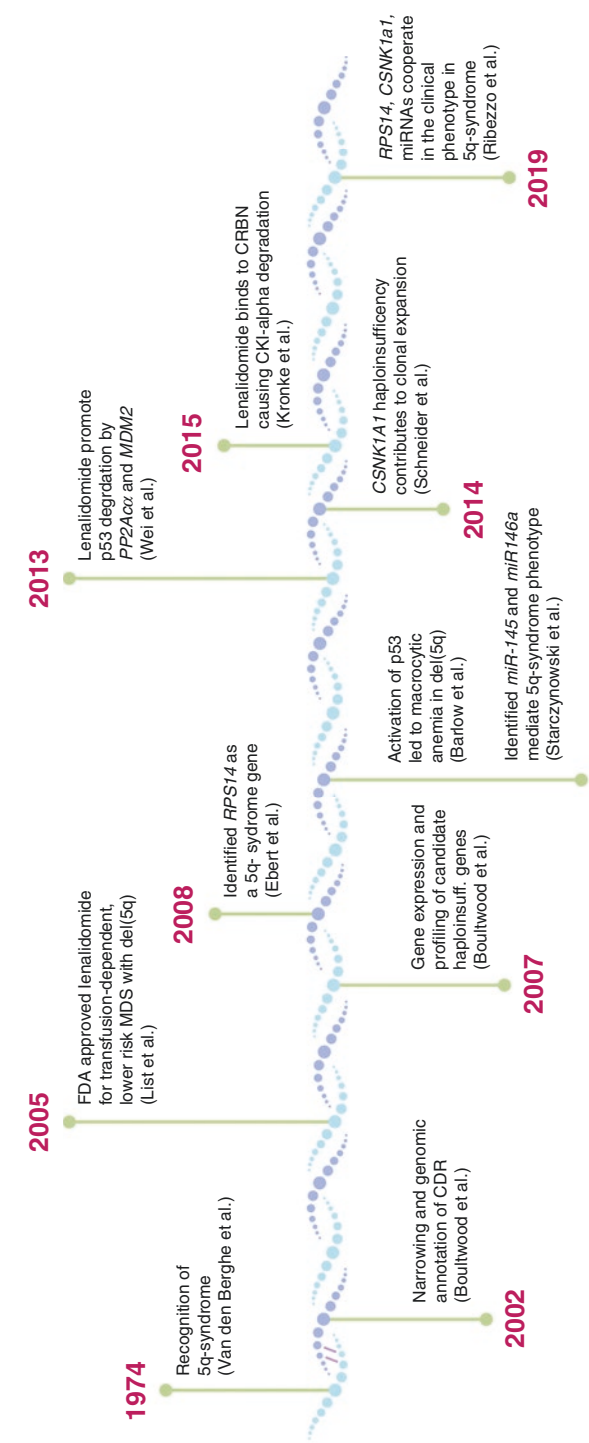

Fig. 3.1 Major milestones in the study and molecular pathogenesis of MDS with del(5q) overtime

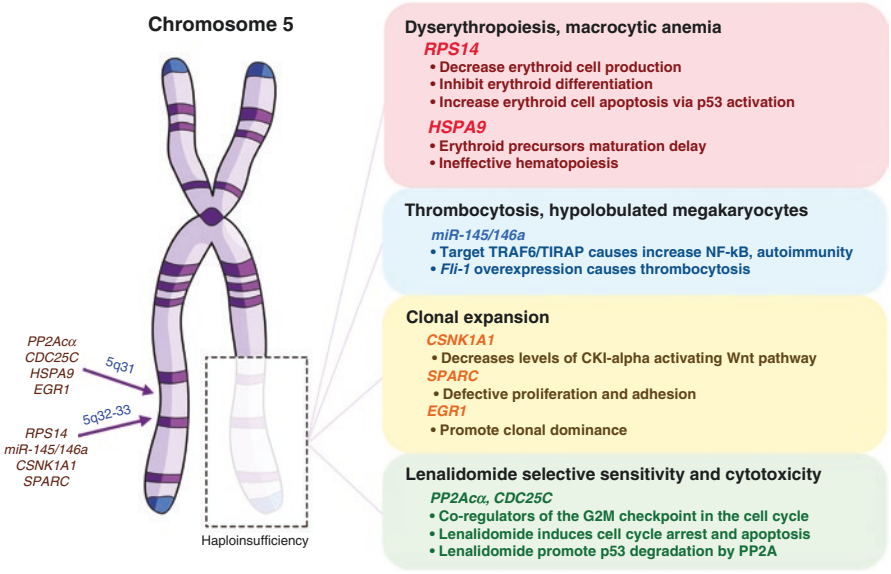

Fig. 3.2 Haploinsufficiency underlies hematologic phenotype, clonal expansion, and lenalidomide selective sensitivity

of differentiating erythroid cells. The level of RPS14 protein expression after knockdown was approximately half that in control cells supporting the hypothesis of haploinsufficiency [19]. In addition, investigators were able to restore erythroid differentiation in bone marrow cells of patients with 5q- syndrome through *RPS14* overexpression, but not in patients lacking del(5q). This established RPS14 deficiency as the principal genetic driver of the dyserythropoiesis in MDS with isolated del(5q) [19]. Interestingly, Diamond–Blackfan anemia, known for its profound erythroid hypoplasia, is a heritable disorder of aberrant ribosome biogenesis caused by haploinsufficiency of a different ribosomal processing gene, *RPS19* [20].

In the last decade, animal studies provided evidence for p53-dependent mechanism in the pathophysiology of the 5q- syndrome [21, 22]. Using large-scale chromosomal engineering, Barlow and colleagues created a mouse model with allelic deletion of the syntenic genes of the human CDR that phenocopied the morphological and hematologic features of the 5q- syndrome [21]. Mechanistically, accumulation of the p53 protein was indispensable for the phenotype, which was validated by crossing the CDR haplodeficient mice with p53-deficient mice, demonstrating complete rescue of the pathologic features. Mouse double minute 2 protein (MDM2) is a key negative regulator of p53 [23]. Free ribosomal proteins (RP) such as RPL11 are liberated as a consequence of *RPS14* haploinsufficiency, binds to MDM2, thereby inhibiting p53 ubiquitination [22]. Activation of p53 induces the programmed death of erythroid precursors ultimately manifest clinically as hypoplastic anemia [24]. In a recent study, Youn and colleagues created a zebrafish model with *RPS14* deficiency that mirrors the anemia phenotype of del(5q) MDS and also demonstrated the induction of matrix metallopeptidase 9 (*MMP9*) expression, a

collagenase known to augment solid tumor growth and invasion, which has been implicated in the initiation and progression of hematological malignancies [25, 26]. Treatment with MMP9 inhibitors partially rescued the erythroid defect. Using a double knockdown technique in human bone marrow progenitor cells, the negative regulatory effect of enhanced *MMP9* expression on erythroid development in *RPS14* knockdown cells was confirmed, supporting its contribution to anemia [26].

Heat shock protein family A (Hsp70) member 9 (*HSPA9*), a gene located in the proximal CDR (5q31.1), was also found to contribute to ineffective erythropoiesis. HSPA9, also known as mortalin, is the only HSP70 homolog localized in the mitochondria matrix that serves as chaperone for the client proteins p53 and S100A9 [27] and has a key role in iron-sulfur (Fe-S) biogenesis [28]. Knockdown of *HSPA9* in the mouse model and in human cells results in erythroid precursor maturation delay, growth arrest, and excess cell death [29]. Liu and colleagues demonstrated that *HSPA9* haploinsufficiency induces overexpression of *TP53*, increased apoptosis, and inhibition of cell growth [30]. However, simultaneous knockdown of *HSPA9* and *TP53* mitigates this effect, suggesting the erythroid defects related to *HSPA9* deficiency is p53-dependent, analogous to the pathogenesis of anemia in del(5q) MDS.

MicroRNA (miRNA) are small, noncoding RNA molecules that post-translationally silence genes by binding to complementary messenger RNAs (mRNA) to direct their degradation. Haploinsufficiency of two miRNA genes, *miR-145* (5q33.1) and *miR-146a* (5q33.3), in the distal CDR are responsible for the other key features of the 5q- syndrome, specifically thrombocytosis, hypolobulated megakaryocytes, neutropenia, and deregulation of the myddosome signaling complex [15, 31]. Starczynowski and colleagues identified two targets of *miR-145/146a*, tumor necrosis factor receptor-associated factor-6 (TRAF6) and Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP), which lies upstream of TRAF6 in Toll-receptor (TLR) signaling [31]. Elevation of TIRAP and TRAF6 activates the TLR-myddosome signaling axis and the downstream inflammatory transcription factor nuclear factor-kappa B (NF-kB), promoting cytokine generation and expansion of the del(5q) clone [32]. Enforced expression of *TRAF6* or *miR-145/146a* knockdown in murine models results in thrombocytosis, megakaryocytic dysplasia, and mild neutropenia [31]. Mice transplanted with *TRAF6*-expressing marrow progressed to either AML or marrow failure. In addition, Friend leukemia virus integration 1 (Fli-1) is a megakaryocyte and erythroid transcription factor that is normally repressed by *miR-145*. *Fli-1* overexpression preferentially expands megakaryocytic progenitors relative to erythroid cells, thereby contributing to thrombocytosis [33].

The epistatic interaction between neighboring haploinsufficient genes, specifically *miR-146a* and TRAF-interacting protein with forkhead-associated domain B (*TIFAB*), may further compound the neutropenia in del(5q) MDS [34]. In one animal study, deleting both *TIFAB* and *miR-146a* led to severe cytopenia mimicking a bone marrow failure-like state. The severity and frequency of leukopenia were less in mice with singular deficiency of either *TIFAB* or *miR-146a* [34]. Ribezzo and colleagues also recently described how combined insufficiency of *RPS14*, *CSNK1A1*, and *miR-145/146a* recapitulate the classic features of 5q- syndrome in a mouse

model [35]. Furthermore, they demonstrated that these deficiencies activate the innate immune response resulting in overexpression of S100A8, an endogenous Toll-like receptor 4 (TLR4) ligand that plays a role in inflammation, in the mesenchymal stem cell niche providing evidence that intrinsic defects of 5q- syndrome directly alters the microenvironment that contributes to ineffective hematopoiesis [35]. Figure 3.3 illustrates how allelic insufficiency drives aberrant innate immune signaling in del(5q) MDS. Collectively, these data indicate that the molecular pathogenesis of the del(5q) MDS hematologic phenotype is dictated by haploinsufficiency of genes encoded within the CDRs.

Haploinsufficiency Underlies Clonal Expansion

CSNK1A1 is a tumor suppressor gene located in the distal CDR (5q32) that encodes Casein Kinase I alpha (CKIα), a regulator of Wnt signaling and stem cell self-renewal [36]. Haploinsufficient *CSNK1A1* reduces levels of the CKIα protein, a component of the β-catenin destruction complex, that binds to and phosphorylates β-catenin. Conditional inactivation of *CSNK1A1* in a murine model showed that haplodeficiency induces hematopoietic stem cell expansion and a competitive repopulation advantage, whereas homozygous deletion induced hematopoietic stem cell failure [37]. Secreted protein acidic and rich in cysteine (*SPARC*) is a haplodeficient candidate tumor suppressor gene found in the CDR that has roles in proliferation and adhesion; however, the precise functional consequence of allelic insufficiency remains unclear [38, 39]. Other genes thought to promote del(5q)

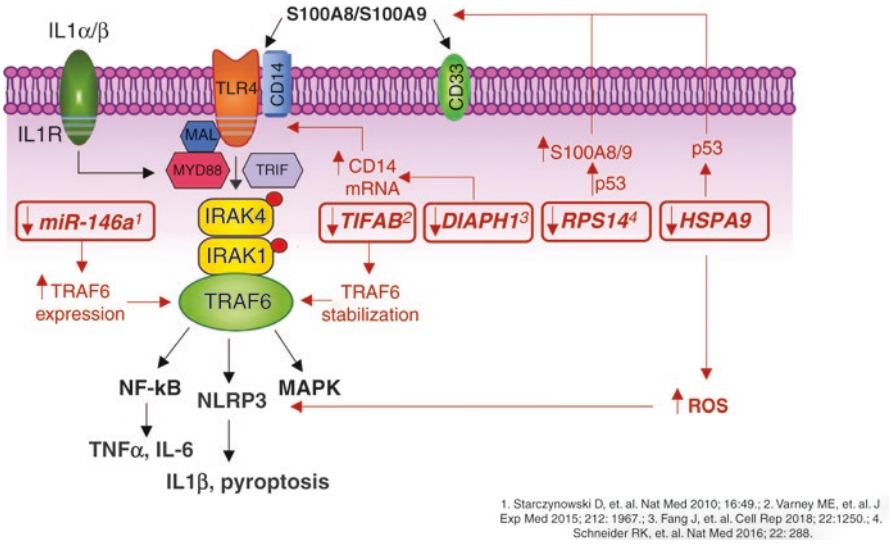

Fig. 3.3 Allelic insufficiency drives aberrant innate immune signaling in del(5q) MDS

clonal dominance include early growth response 1 (*EGR1*), located in the proximal CDR and adenomatous polyposis coli (*APC*) [40]. In one study, haploinsufficiency of EGR1 and the loss of TP53 in HSC compounded the rate of hematologic neoplasm development [41]. Similarly, deficiency in both EGR1 and APC cooperate in the presence of *TP53* deficiency to promote AML transformation [41].

Lenalidomide in Del(5q) MDS and Its Mechanism of Action

Besides the distinct clinical phenotype, MDS with del(5q) is unique among other subtypes of MDS because of its selective sensitivity to lenalidomide, a thalidomide analog. The karyotype-specific activity was first observed int he dose-finding study (MDS-001) where patients with del(5q) lesion had greater response rate (83%) compared to patients with other karyotypes (57% for patients with normal karyotype and 12% with other karyotypes, $p = 0.007$) and led to clonal suppression and cytogenetic response [42]. This led to the pivotal MDS-003 phase II clinical trial which included transfusion-dependent patients with a del(5q) lesion and low/intermediate-1 (int-1) risk disease according to IPSS [43]. Among 148 patients treated, 76% had a 50% or greater reduction in transfusion needs, and 67% achieved transfusion independence with a median rise in hemoglobin of 5.4 g/sl, providing the basis for its approval by the Food and Drug Administration (FDA) in 2005. At the time of drug approval, the mechanism of action of lenalidomide was not fully delineated; however, significant progress has been made since that time.

Lenalidomide is an immunomodulatory drug found to selectively inhibits del(5q) clones through several mechanisms. The dual specificity phosphatases, CDC25C (cell division cycle 25C) and PP2Acα (protein phosphatase 2A catalytic domain alpha) encoded within or adjacent to the proximal CDR are important co-regulators of the G2M checkpoint in the cell cycle [44]. Wei and colleagues showed that cells with reduced expression of *CDC25C* and *PP2Ac*α have enhanced sensitivity to lenalidomide, which causes G2M cell-cycle arrest and induction of apoptosis [45]. Lenalidomide inhibits phosphatase activity directly and indirectly. Lenalidomide inhibition of haplodeficient PP2A stabilizes MDM2 by hyperphosphorylating inhibitory residues, thereby promoting p53 degradation [46]. Kronke and colleagues in a proteomic study showed that haploinsufficient *CSNK1A1* cells are sensitized to lenalidomide, which makes these cells even more vulnerable due to additional degradation of CKIα [47]. Specifically, lenalidomide binds to cereblon (CRBN), the substrate receptor of the CRL4-CRBN E3 ubiquitin ligase and induces recruitment of the CKIα (substrate) [48]. Regulator of Cullin 1 (ROC1) serves to recruit the E2 enzyme that binds to ubiquitin. Ubiquitination and subsequent degradation of CKIα led to del(5q) progenitor cell arrest and death [49]. Furthermore, overexpression of *CSNK1A1* reduced the sensitivity of lenalidomide only in the HSC of patients with del(5q) MDS and not those with normal cytogenetics [50]. Taken together, the multipronged approach of lenalidomide in del(5q) MDS explains the high selectivity and efficacy in this karyotypically defined MDS subset (Fig. 3.4).

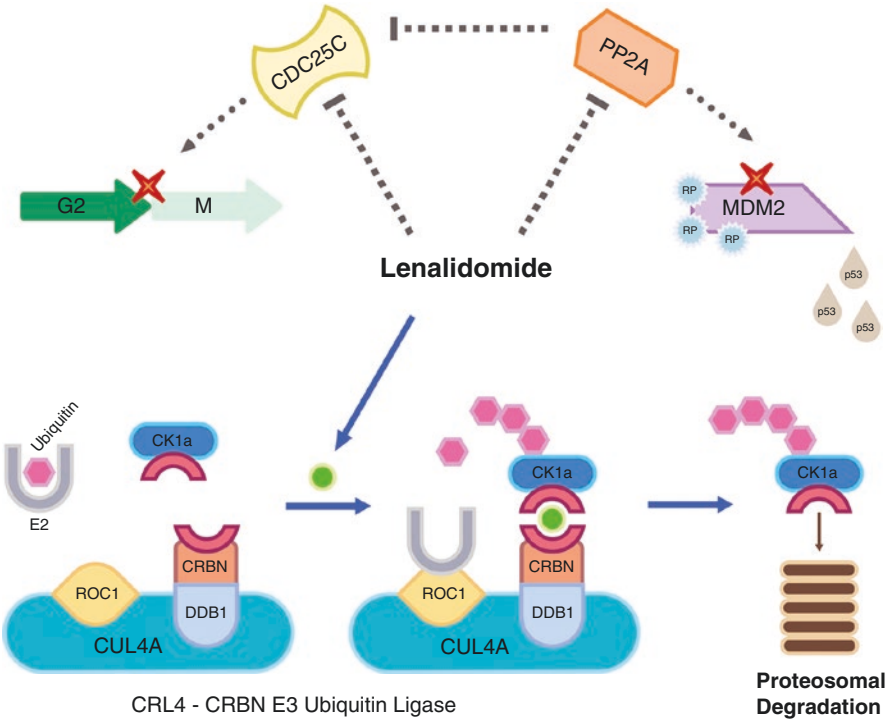

Fig. 3.4 Lenalidomide mechanism of action in del(5q) MDS. Reduced expression of cell division cycle 25C (CDC25C) and protein phosphatase *2A* catalytic domain alpha (PP2Ac*α*) enhances sensitivity to lenalidomide. Lenalidomide directly inhibits CDC25C inducing G2M cell cycle arrest and apoptosis in malignant cells (top left). It also inhibits PP2Ac*α* resulting in mouse double minute 2 protein (MDM2) stabilization and subsequently p53 degradation (top right). Recall it is the haploinsufficient ribosomal processing S14 (*RPS14*) that produces free ribosomal proteins (RP) that bind to MDM2 causing pathologic p53 accumulation. Inhibiting PP2A also causes CDC25C inactivation. Lenalidomide thereby selectively eliminate del(5q) cells and restore effective erythropoiesis. In addition, it binds to cereblon (CRBN), the substrate receptor of the CRL4-CRBN E3 ubiquitin ligase that is composed of damaged DNA-binding protein1 (DDB1), cullin 4a (CUL4A), and regulator of cullins 1 (ROC1) (bottom left). It induces recruitment of CKI*α* (substrate). Ubiquitin-conjugating enzyme (E2) functions with ROC1 to facilitate ubiquitin transfer to the substrate or ubiquitin chain (bottom middle) resulting in ubiquitination and degradation of CKI*α* (bottom right)

Mechanism of Lenalidomide Resistance in Del(5q) MDS

While most patients with lower-risk del(5q) MDS achieve remission with lenalidomide, the median duration of response is approximately 2.5 years [51, 52]. To determine if persistent malignant stem cells are responsible for relapse, Tehranchi and colleagues investigated specimens from 7 patients with del(5q) MDS who achieved a complete cytogenetic remission with lenalidomide [53]. Lenalidomide was able to

selectively and generally complete deplete del(5q) progenitor cells (CD34+, CD38+); however, a phenotypically distinct, quiescent group of del(5q) stem cells (CD34+,CD38−/low, CD90+) persisted. As the del(5q) clone expands over time under the selective pressure of lenalidomide, resistance develops and recurrence occurs [53]. In a separate study, *PP2Acα* overexpression induces resistance to lenalidomide, resulting in suppression of MDM2 and accumulation of p53 [46]. Strong p53 expression by immunohistochemistry (IHC) in turn is associated with *TP53* mutation, which can occur in about 18% of low-risk MDS del(5q) patients [54]. Furthermore, Saft and colleagues examined bone marrow specimens of 85 patients with IPSS low or int-1 risk del(5q) from the MDS-004 trial who were treated with lenalidomide and found strong p53 expression correlated with higher rates of AML transformation ($p = 0.0006$), lower rates of cytogenetic response ($p = 0.009$), and decreased overall survival ($p = 0.0175$) [55]. Cells with strong p53 expression were confirmed to have *TP53* mutation by pyrosequencing analysis. Developing novel medications targeting these defects is an area of active research and promising therapeutics such as cenersen (works to reduce cellular p53) and APR-246 (refold mutant p53 back to its wild-type conformation) is currently under investigation [56, 57].

Conclusion

Over the past decade, new insights into the pathogenesis of MDS with del(5q) have unveiled how allelic haploinsufficiency gives rise to the distinctive clinical phenotype. Haploinsufficient *RPS14*, *miR-145/146a*, and *CSNK1A1* located in the distal CDR and *HSPA9* and *EGR1* in the proximal CDR are epistatic molecular contributors to the hematologic manifestations of the disease. The selective sensitivity of lenalidomide in the del(5q) clone arises from CKI*α* degradation by binding to CRBN, and CDC25C and PP2A phosphatase inhibition. Understanding these mechanisms of lenalidomide action also sheds lights into the mechanisms of resistance. Much work is still needed to discern strategies to circumvent resistance and additional opportunities for therapeutic intervention.

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