Chapter 5
Molecular Landscape of MDS

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Introduction

In the last decade, the advance of next-generation sequencing (NGS) has greatly expanded our insight into the underlying pathobiology of myelodysplastic syndromes (MDS). The contribution of cytogenetic aberrations to MDS was realized early on and has been implemented into classification, prognostication, and treatment planning [1–3]. However, only approximately half of MDS patients have a detectable cytogenetic aberration [4–8]. On the other hand, large-scale studies identified molecular genetic abnormalities in up to 80–90% of patients with de novo MDS [9, 10]. Mutations recurrent in MDS can also be found in other myeloid or – to a lesser extent – lymphoid neoplasms, albeit at varying frequencies [11].

Categorized according to their biological function, mutations can be assigned to one of seven major classes (compare Table 5.1).

Table 5.1 Overview of dysregulated pathways and biological processes in MDS

Class	Affected pathway	Effect
DNA methylation	Epigenetic regulation	Transcriptional dysregulation
Histone modification		
Transcription factors	Transcription	
Cohesin components	DNA looping	
Splicing factors	Splicing	Post-transcriptional dysregulation
Signaling factors	Signaling	Aberrant proliferation
p53 pathway factors	p53 pathway	Genetic instability Aberrant activation of DNA damage response Disruption of cell cycle control

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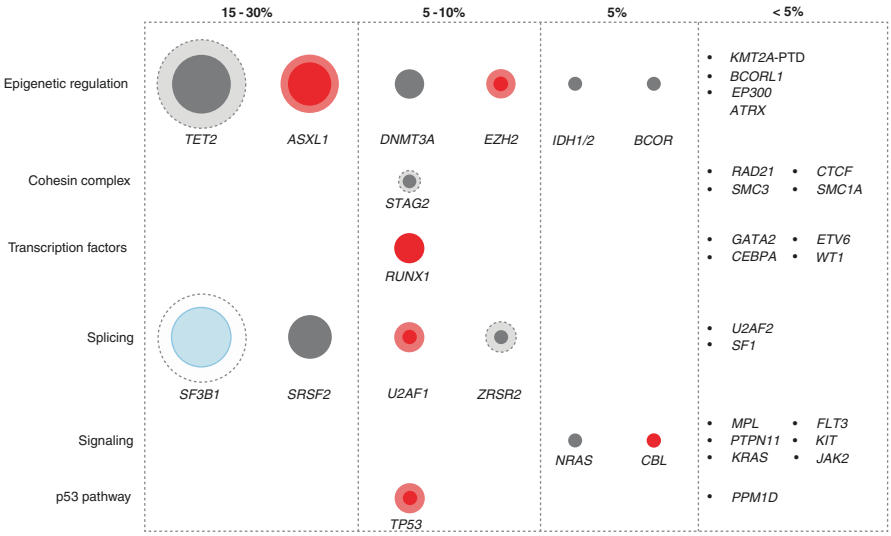

Fig. 5.1 Recurrently mutated genes in MDS, categorized according to biological function and mutation frequency. Circle size correlates with mutation frequency, light colored halos indicate the upper limit of frequency. Genes are mutated at frequencies ≥5% according to [12]. Genes mutated in less than 5% of cases are listed as bullet points, selection according to [9, 10]. Mutations that confer an IPSS-R-independent negative effect are colored in red/light red, mutations with no clear independent effect are displayed as gray/light gray circles. Only *SF3B1* mutations are associated with a favorable prognosis (light blue). Prognostic relevance according to [13]

Recurrently Mutated Genes in MDS

Figure 5.1 gives an overview of frequently mutated genes in MDS. Molecular aberrations with mutation frequencies ≥5% will be discussed in greater detail below.

Molecular Aberrations Contributing to Transcriptional Dysregulation in MDS

Epigenetic Regulation

Epigenetics is a major contributor to the regulation of gene expression. Based on the signature of epigenetic marks, which in human cells comprise DNA methylation and histone modifications, genes are either in a repressed or active state. However, this is no binary phenomenon, and the expression strength of active genes is tightly regulated. Setting epigenetic marks is an adaptive and reversible process and requires "writers" and "erasers." The epigenetic signature is recognized by "readers," which directly or indirectly mediate the biological outcome of the respective

epigenetic signature [14]. Epigenetic dysregulation is a hallmark of cancer since it allows tumor cells to silence tumor suppressor genes, activate or overexpress oncogenes, and to reset or halt cell differentiation [15, 16].

DNA Methylation

DNA methyltransferases (DNMT) can transfer a methyl group to the 5′ carbon of cytosine in CpG dinucleotides and thus belong to the class of epigenetic writers [17]. Erasure of DNA methylation is initiated by ten-eleven-translocation 2 (TET2), a methylcytosine dioxygenase. TET2 is thought to catalyze the first demethylation step, that is, the conversion of 5-methylcytosine to 5-hydroxymethylcytosine [18] (see Fig. 5.2). Both hypomethylation and hypermethylation phenotypes can contribute to pathobiology [17], the latter, however, can be pharmacologically antagonized with hypomethylating agents (HMA). The extensive methylation of promotor regions is strongly associated with gene silencing and malignant cells exploit this property to silence (putative) tumor suppressor genes, especially in high-risk MDS. The therapeutic effect of HMA appears to be greatly attributed to the reactivation of these genes [16].

DNMT3A *DNMT3A* mutations can be found in ~10% of MDS patients [12], however, they also represent the single most frequent aberration associated with age-related clonal hematopoiesis of indeterminate potential (CHIP) [19–21]. *DNMT3A* mutations themselves are not considered sufficient to drive MDS pathogenesis, but they contribute to gene expression deregulation by aberrant DNA methylation. Up

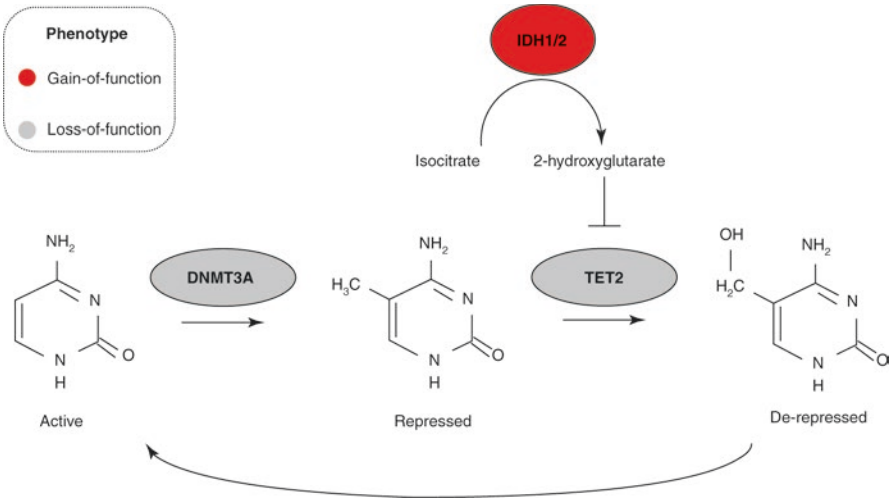

Fig. 5.2 Recurrent mutations in *DNMT3A*, *TET2*, and *IDH1/2* in MDS affect DNA methylation and contribute to an aberrant epigenome by causing a hypo- or a hypermethylation phenotype

to ~50% of *DNMT3A* mutations in MDS affect the arginine at position 882 [22–25]. A *DNMT3A*-R882H mutation leads to a loss-of-function phenotype and a decrease of catalytic activity by 80% [26]. Moreover DNMT3A-R882H exerts a dominant negative effect on wildtype DNMT3A, which adds to the loss-of-function phenotype [26]. *DNMT3A* mutations in MDS are associated with inferior overall survival and higher risk of transformation in many but not all studies [22–25, 27–30].

TET2 In accordance with the role of wildtype TET2 as eraser of DNA methylation, loss-of-function mutations of the *TET2* gene result in aberrant DNA hypermethylation [16, 31]. In MDS, up to 30% of patients have a detectable *TET2* mutation [12]. *TET2* mutations are also recurrently detected in other myeloid neoplasms as well as in CHIP, where the mutation frequency is approximately 10% [19–21]. While found associated with favorable outcome in one study [32], several other studies could not establish any influence of *TET2* mutations on prognosis [33–35].

IDH1/2 Isocitrate dehydrogenases IDH1 and IDH2 are predominantly known for their role in the tricarboxylic acid cycle, where they catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. Mutations of *IDH1* exclusively affect the arginine (R) at position 132, and in *IDH2* either codon R140 or R172 is found mutated [16]. Mutations in *IDH1/2* lead to a gain-of-function phenotype, since isocitrate is converted to 2-hydroxygluturate, instead of 2-oxoglutarate [36]. This aberrant metabolite competitively inhibits 2-oxoglutarate-dependent enzymes, including TET2 [16, 37, 38] (see Fig. 5.2). Accordingly, *IDH1/2* mutations are associated with a DNA hypermethylation phenotype [39]. The prognostic importance of *IDH1/2* mutations in MDS is unclear due to contradicting data [40, 41].

Histone Modifications

Histones, once thought of as merely "packaging material" for DNA, provide a versatile and highly dynamic platform for a myriad of different post-transcriptional modifications that fine-tune gene expression [15]. The concrete effect of histone modifications on transcription depends not only on the individual type of modification (e.g., acetylation, methylation, and ubiquitination) but also on the number and specific position of histone marks and the combinatorics of histone modifications (known as histone code) [15, 42]. Histone methylation, which can take the form of mono-, di-, and trimethylation, can represent either a repressive or an activating epigenetic mark. Here, a variety of "readers" are dedicated to interpret the respective methylation mark. For example, trimethylation of histone 3 lysine 4 is an "active" mark, while trimethylation of histone 3 lysine 27 (H3K27me3) is an "inactive/suppressive" epigenetic mark [15, 43].

H3K27me3 and its downstream effects are recurrently dysregulated in MDS. Under physiological conditions, H3K27me3 is "written" by the polycomb repressive complex 2 (PRC2), whose catalytic subunit is EZH2. Polycomb repressive complex 1 (PRC1) is both "reader" and "writer" at the same time. Upon recruitment

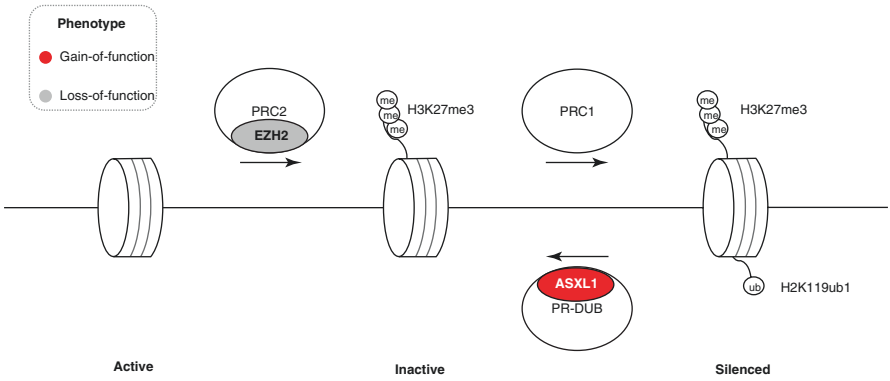

Fig. 5.3 Polycomb repressive complexes (PRC) contribute to transcriptional gene silencing by establishing the repressive epigenetic marks H3K27me3 (by PRC2) and H2K119ub1 (by PRC1). The PR-DUB complex can antagonize the action of PRC1. Mutations in *EZH2* and in *ASXL1* are found recurrently in MDS and result in an aberrant histone code

to H3K27me3, PRC1 marks histone H2A at lysine 119 with an ubiquitin molecule, and H2AK119 monoubiquitination (H2AK119ub1) results in further chromatin compaction and transcriptional silencing [43, 44]. The H2AK119ub1 mark can be erased by the polycomb repressive deubiquitinase (PR-DUB) complex [43, 44], in which ASXL1 functions as a chromatin binding subunit [45] (see Fig. 5.3).

EZH2 Mutations in the histone methyltransferase *enhancer of zeste 2* (*EZH2*) gene lead to loss of function by abrogating or strongly diminishing EZH2 catalytic activity and thus to impaired silencing by the PRC2 complex [46, 47]. Patients with *EZH2* mutations have a poor prognosis [13, 47], independent from IPSS-R [13].

ASXL1 Wildtype additional sex combs-like 1 (ASXL1) interacts with a variety of proteins; among other functions, it facilitates recruitment of PRC2 to target loci by protein–protein interactions with PRC2 subunits [48]. As mentioned above, ASXL1 is also part of the PR-DUB complex. The nonsense or frameshift mutations observed in myeloid neoplasms lead to truncated ASXL1 protein, which is thought to gain in function. Truncated ASXL1 hyperactivates the PR-DUB complex [49, 50], and in contrast to wildtype ASXL1 it interacts with BRD4, an epigenetic reader, which promotes transcriptional activation [51, 52]. Ultimately, mutations in *ASXL1* cause aberrant gene expression. *ASXL1* mutations are associated with a IPSS-R-independent poor prognosis [13, 53]. They are also found in ~9% of individuals with CHIP [19–21].

BCOR Aside to its name giving function as BCL6 corepressor (BCOR), BCOR is a subunit of a variant polycomb repressive complex 1, called PRC1.1. In contrast to the "canonical" PRC1 complex, PRC1.1 ubiquitinates loci independent from preset H3K27me3 marks [54]. The physiological function of the PRC1.1 complex appears to be the maintenance of a pluripotent state in stem cells. Mutations in *BCOR* thus lead to differentiation dysregulation and contribute to pathobiology [54, 55]. *BCOR* mutations are associated with a poor prognosis [56].

DNA Looping

Cohesins are named for their essential function in sister chromatid cohesion. A multiprotein ring-shaped complex consisting of STAG2, RAD21, SMC3, and SMC1A stabilizes the sister chromatids during metaphase and prevents replication fork collapse [11]. Moreover, the cohesin complex is now known to mediate interaction between distant genomic loci (e.g., promoter and its distant enhancer) by stabilization of DNA loops [57] (compare Fig. 5.4). It appears that dysregulation of cohesin-mediated DNA looping contributes to MDS pathogenesis through alteration of gene expression, since cohesin mutations in MDS are not associated with chromosomal aberrations [58]. In MDS, *stromal antigen 2* (*STAG2*) is the most frequently mutated cohesin [9, 10], and represents a poor prognostic marker [10, 58, 59].

Transcription Factors (TF)

Transcription is a well-orchestrated cellular process in which general transcription factors enable transcription and specific transcription factors regulate gene expression. Specific transcription factors themselves are tightly regulated by expression in a cell-type specific and/or temporal manner. Moreover, they are dedicated to the regulation of a specific set of target genes [60]. The core binding factor (CBF) family of proteins, for example, are master regulators of hematopoietic ontogeny and differentiation [61]. *Runt-related transcription factor 1* (*RUNX1*), which encodes the DNA-binding α-subunit of the heterodimeric CBF, is the most frequently mutated TF gene in MDS. *RUNX1* mutations are associated with a poor prognosis [10, 13, 62], independent from IPSS-R [13]. Moreover, individuals with a *RUNX1* germline mutation have an increased risk of developing myeloid neoplasms. This also holds true for germline mutations of the TF genes *CEBPA*, *ETV6*, and *GATA2*, which all define "myeloid neoplasms with germline predisposition" in the WHO classification [12]. Somatic mutations of *CEBPA*, *ETV6*, and *GATA2* are also found in MDS with mutation frequencies <5% [10].

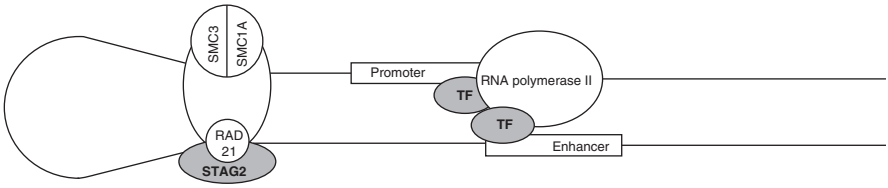

Fig. 5.4 Transcriptional dysregulation in MDS can result from abnormalities in cohesin complex-mediated DNA looping, which allows to bring distant gene regulatory elements (such as enhancer and promoter) into spatial proximity. Among the cohesins, *STAG2* is the most frequently mutated gene (gray). Transcription by RNA polymerase requires general transcription factors and is regulated by specific transcription factors that bind to regulatory elements (e.g., enhancer and promoter). Transcription factor (TF) mutations in MDS are found recurrently (gray) in master regulators of the hematopoietic cell differentiation program, for example, in the *RUNX1* gene

Molecular Aberrations Contributing to Dysregulation of Splicing

Following transcription, pre-mRNAs undergo a number of maturation steps, among them is splicing, that is, the removal of non-coding "intronic" sequences. The modular structure of metazoan pre-mRNAs, consisting of coding (exonic) and non-coding (intronic) sequences, is the prerequisite for alternative splicing, i.e., the selective inclusion or exclusion of a given exon. Due to alternative splicing, several protein isoforms can be generated from the same gene sequence, resulting in a complex proteome.

Splicing is a well-orchestrated, multi-step process catalyzed by the spliceosome, whose composition changes during the splicing process, making different subcomplexes distinguishable. Up to 60% of MDS patients carry a mutation in a splicing factor [63–66]. Most interestingly, splicing mutations in MDS mainly affect early spliceosome assembly at the 3′ splice site [11], see also Fig. 5.5.

Spliceosome formation is promoted by SR proteins, which are named after a protein domain that is enriched in serine (S) and arginine (R) and binds to exonic splicing enhancers. In MDS, *SRSF2*, which encodes such an SR protein, is found recurrently mutated [12, 67].

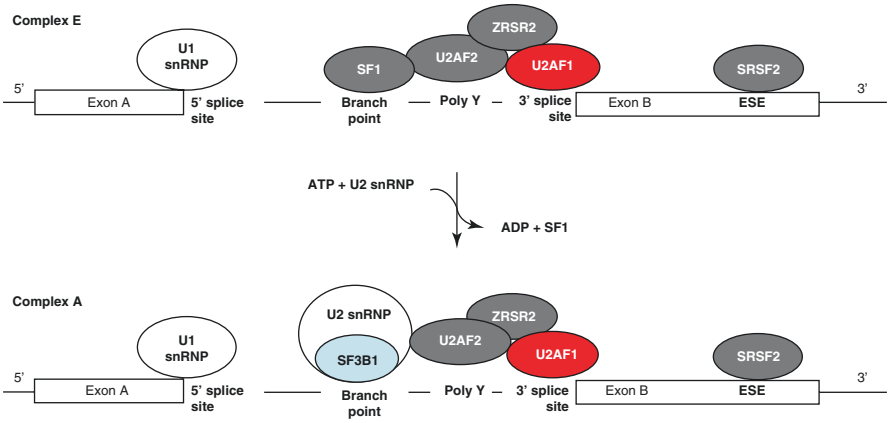

Fig. 5.5 Early spliceosome assembly is promoted by recognition of exonic splicing enhancers (ESE) by SR proteins. Correct positioning of splicing factors at regulatory intronic and exonic sequences is integral to the splicing process. U1 snRNP is required for the recognition of the 5′ splice site, while the 3′ spliceosome is composed of multiple factors. In complex E (commitment complex) splicing factor 1 (SF1) binds to the branch point region. The U2 auxiliary complex, comprised of U2AF1 and U2AF2, recognizes the 3′ splice site and the polypyrimidine tract, respectively (Y = pyrimidine). The transition to complex A (pre-spliceosome) is an energy-dependent step and leads to displacement of SF1 and the recognition of the branch point region by the U2 snRNP through its RNA binding subunit SF3B1. Factors found recurrently mutated in MDS are color-coded. Gray: factors without an independent prognostic value; red: *U2AF1* mutations are associated with a poor prognosis, independent from IPSS-R; light blue: mutations in *SF3B1* confer a favorable prognosis. Prognostic relevance according to [13]

Table 5.2 Interplay between *cis* (i.e., sequence) elements and *trans* (i.e., protein) factors to enable precise recognition and definition of exon-intron boundaries

Sequence	Recognized by
Complex E (commitment complex)	
5′ splice site	U1 snRNP
Branch point region	Splicing factor 1 (SF1)
Polypyrimidine tract	U2 auxiliary factor 2 (U2AF2)
3′ splice site	U2 auxiliary factor 1 (U2AF1)
Complex A (pre-spliceosome)	
5′ splice site	U1 snRNP
Branch point region	SF3B1 (as RNA binding subunit of U2 snRNP)
Polypyrimidine tract	U2 auxiliary factor 2 (U2AF2)
3′ splice site	U2 auxiliary factor 1 (U2AF1)

Correct splicing requires precise definition of exon-intron boundaries, which is facilitated by recognition of specific intronic and exonic sequences by dedicated factors (compare Table 5.2).

Mutations in *SF3B1*, *SRSF2*, and *U2AF1* alter the binding preferences of the respective encoded splicing factor, while mutations in *ZRSR2* result in complete loss of activity [67].

SF3B1 Mutations in *splicing factor 3b subunit 1 (SF3B1)* are strongly associated with a ring sideroblast (RS) phenotype, caused by aberrant accumulation of iron in mitochondria. The majority of MDS-RS patients carry a *SF3B1* mutation [63, 64, 68]. The *SF3B1* mutational status influences classification according to WHO (2017): in cases with wildtype *SF3B1*, ≥15% ring sideroblasts (as percentage of bone marrow erythroid elements) are required for the diagnosis of MDS-RS, however, if *SF3B1* is mutated, ring sideroblasts between 5% and 14% are sufficient [12]. Among splicing factors, it is also the only mutation that is associated with a favorable prognosis [13, 63, 68, 69].

SRSF2 Mutations in the *serine- and arginine-rich splicing factor 2* (*SRSF2*) gene are associated with a poor prognosis [70]. As is the case for *SF3B1* and *U2AF1* mutations, *SRSF2* mutations are heterozygous missense mutations and occur in distinct hotspots [11, 67, 71]. As a consequence, the binding preference of SRSF2 is altered, leading to an aberrant exonic enhancer site-binding pattern [67]. By this mechanism, mutations in *SRSF2* cause e.g. mis-splicing and aberrant degradation of *EZH2* transcripts, indirectly contributing to an aberrant epigenome [72].

U2AF1 *U2 small nuclear RNA auxiliary factor 1* (*U2AF1*) mutations confer an inferior prognosis independent from IPSS-R [13]. Given the importance of U2AF1 for the recognition of intron-exon boundaries, mutations that affect binding preferences result in increased exon skipping [11, 67].

ZRSR2 In "constitutive" splicing by the major spliceosome, ZRSR2 (zinc finger CCCH-type, RNA binding motif, and serine/arginine rich 2) interacts with the U2AF complex and stabilizes the formation of complex A [73]. However, a subset of transcripts of 700 to 800 genes are spliced by the "minor" spliceosome, in which ZRSR2 assumes the functional role of the U2AF complex [73–75]. *ZRSR2* mutations are thought to contribute to MDS disease biology by aberrant intron retention and mis-splicing in minor spliceosome-dependent transcripts [76]. In contrast to other splicing factors, mutations in *ZRSR2* do not occur in distinct hotspots [71]. The outcome and clinical course of patients with *ZRSR2* mutations is strongly dependent on *TET2* mutational status. Cases with mutated *ZRSR2* and wildtype *TET2* were observed to have a high AML transformation rate and a poor prognosis [71].

Molecular Aberrations Contributing to Dysregulation of Signaling

In comparison to other myeloid neoplasms, mutations in signaling factors are less common in MDS. Signaling factor mutations in AML are considered to represent late events and as such are often associated with progressive disease when found in MDS. Most frequently, the MAP kinase pathway is affected in ~10% of MDS patients [9–11]. *NRAS (neuroblastoma RAS viral oncogene homolog)*, which encodes one factor of this pathway, is found mutated in ~5% of MDS patients [12]. Mutations in *CBL (casitas B-lineage lymphoma)*, which are also detected in ~5% of MDS patients [12], are more prevalent in chronic myelomonocytic leukemia (CMML). Both gene mutations are linked to an inferior prognosis [9, 13, 53, 77–79], in case of *CBL* independent of IPSS-R [13]. Moreover, mutations in *CBL* are associated with aberrantly prolonged activation of other signaling factors, for example, FLT3 [80]. Mutations in the *FLT3* gene rarely occur in MDS; however, if present, they are associated with a very poor prognosis and progression to secondary AML [81–83].

Molecular Aberrations Contributing to Dysregulation of the p53 Pathway

Aberrations that affect the tumor protein p53, also often referred to as "guardian of the genome" are recurrently found in every cancer type. Its physiological function is to halt the cell cycle in case of cellular stresses or DNA damage and to promote, if necessary, apoptosis [11, 84]. Alterations in *TP53*, the gene encoding p53, are caused by deletion or gene mutation. *TP53* deletion is frequent among cases with

deletion of chromosome arm 17q and is commonly accompanied by *TP53* mutation of the other allele [85, 86], resulting in biallelic inactivation and a particularly inferior outcome [87]. *TP53* aberrations are associated with several predictors of poor clinical outcome, such as low platelet count, high blast count, high-risk disease, complex karyotype, and resistance to therapy [53, 88, 89]. The presence of *TP53* aberrations is a negative prognostic factor, independent from IPSS-R [13]. The negative prognostic impact is retained also in the setting of allogeneic stem cell transplantation [11, 29, 90].

In de novo MDS cases, *TP53* alterations are detected in ~5% of patients [12]. In the context of therapy-associated MDS, *TP53* aberrations are found in up to 33% [11, 91]. *TP53* and *PPM1D*, which encodes a phosphatase that negatively regulates p53, have been found mutated in CHIP, with frequencies of ~4% [20, 21]. This finding provides one possible explanation for the development of therapy-associated neoplasms (t-MN). Under the selective pressure of cytotoxic therapy, clones carrying aberrations of *TP53* and/or *PPM1D* gain selective advantage and can undergo clonal expansion. Screening patients for *TP53* and *PPM1D* aberrations prior to cytotoxic therapy could help identify individuals at risk to develop t-MN [92–95].

The Clinical Value of Molecular Genetic Characterization in MDS

Currently, only *SF3B1* mutations are considered as a diagnostic criterion in the WHO classification [12]. Given the diagnostic challenge of cytomorphological evaluation of (subtle) dysplastic features and the low reproducibility of blast count determination, it is likely that molecular genetics will gain in importance in classification in the future. Today, molecular characterization already plays a crucial role in state-of-the-art prognostic evaluation and therapeutic decision making.

Prognosis None of the prognostic models in MDS, discussed in depth in Chap. 7, takes molecular aberrations into account. However, mutations in several genes have been shown to have prognostic power independent from the revised IPSS score (IPSS-R). Aberrations of *ASXL1*, *CBL*, *EZH2*, *RUNX1*, *TP53*, and *U2AF1* have all been associated with significantly shortened overall survival in a study with >3000 MDS patients. Detection of a mutation in one of the six genes should warrant placing a case in the next unfavorable IPSS-R risk group [13].

Therapy Decisions The response to hypomethylating agents is strongly influenced by a patient's mutational landscape, especially in genes encoding epigenetic factors. Azacitidine resistance has been observed in the context of *DNMT3A*-R882 mutations as well as for mutations that affect the SKI domain of *SETBP1*, which also encodes an epigenetic regulator [96]. Another study found that mutations in *ASXL1* and *ETV6* are associated with short response duration [97]. In contrast, patients with *TET2* mutation (in the absence of a concomitant *ASXL1* mutation) showed a particu-

larly high sensitivity to azacitidine [34, 98–100]. However, there were no significant differences in overall survival and response duration between patients with mutated and wildtype *TET2* under azacitidine treatment [98, 100].

The mutation status of *TP53* should play a role in therapy planning in several respects. In general, patients with isolated 5q deletion benefit from treatment with lenalidomide. However, the presence of a concomitant *TP53* mutation poses the risk of faster disease progression [101], therefore *TP53* mutational status should be determined prior to lenalidomide therapy [102]. Moreover, AML and MDS patients with *TP53* mutation have been found to show a better initial response to a 10-day decitabine protocol than to conventional chemotherapy [103]; however, remission was eventually lost in all *TP53* mutated cases, including the nine MDS patients carrying *TP53* mutations. Although patients with *TP53* abnormalities should be considered for allogeneic stem cell transplantation [104], the negative prognostic effect persists post-transplant [11, 29, 90]. In patients eligible for allogeneic transplantation, the *TP53* mutation status should be taken into account for the selection of the conditioning scheme, since patients with *TP53* mutation do not benefit from myeloablative conditioning [105]. Therefore, whenever possible, alternative conditioning regimen should be considered for this patient group, possibly within a study setting [104].

From Clonal Hematopoiesis to Secondary AML – A Disease Continuum?

The advance of NGS led to the coincidental finding of leukemia-associated gene mutations as drivers of clonal hematopoiesis in the absence of hematological disease. CHIP is now known to be an age-related phenomenon [19–21, 106], whose clinical implications remain subject to discussion and research. Only 0.5–1% of individuals with CHIP develop myeloid neoplasms later on [19, 21]. As described above, mutations in three genes are strongly associated with CHIP: *DNMT3A*, *TET2*, and *ASXL1*.

Clonality has also been demonstrated in a major subset of patients with unexplained cytopenia [107, 108]. The presence of gene mutations as clonal drivers was associated with ~14-fold higher risk of progression to myeloid neoplasms compared to cases with idiopathic cytopenia [107]. Accordingly, clonal cytopenia of undetermined significance (CCUS) has been introduced as a pre-malignant condition [107–109].

The recognition of CHIP and CCUS as well as insight into the genetic landscape of MDS validates the multi-hit hypothesis in MDS pathogenesis (compare Fig. 5.6). In MDS, 3 mutations were detectable in the median [0–12 mutations] [10]. Mutations affecting DNA methylation and splicing factors show a higher mutational load than mutations in histone modifiers and signaling factors, which makes early and late mutational events in MDS pathogenesis distinguishable [9, 10].

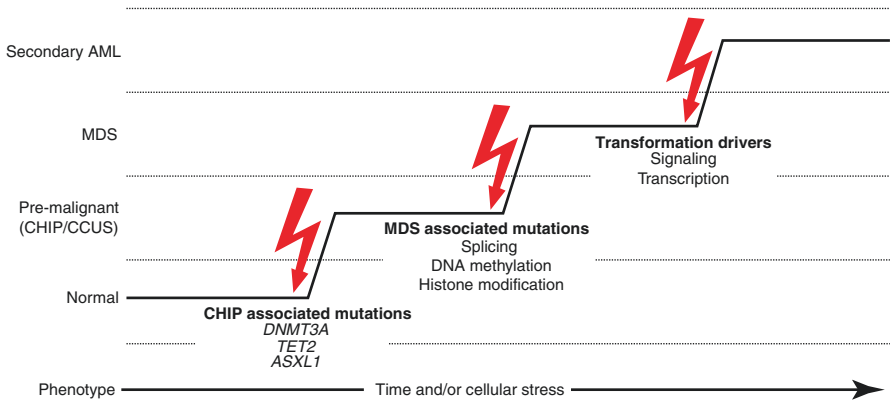

Fig. 5.6 Multi-hit hypothesis in the pathogenesis of myeloid neoplasms. Mutations found associated with CHIP and CCUS are not sufficient for MDS pathogenesis, however, they lay the foundation. Acquisition of additional mutations and/or selective pressure can cause clonal evolution and ultimately lead to the development of myeloid neoplasms

Progression of MDS to secondary AML is associated with abrogation of hematopoietic differentiation and/or uncontrolled proliferation [11]. Mutations in transcription factor genes such as *RUNX1*, *CEBPA*, and *GATA2* often herald disease progression [11, 110]. Same holds true for mutations affecting signaling, especially mutations of RAS pathway factors or *FLT3* are linked to progression to AML [11, 110, 111]. It is of clinical importance to distinguish between cases with sAML and de novo AML, since patients with sAML have an inferior prognosis and often are refractory to chemotherapy [11, 110].

In conclusion, NGS-based panel testing has paved the way for a comprehensive description of the molecular landscape in MDS within just a decade. Panel testing in MDS is increasingly used to support or exclude a diagnosis of MDS in cases of unclear cytopenia(s) and/or dysplasia. Several publications have demonstrated the clinical utility of NGS screening using a panel of genes whose mutation status can inform differential diagnostics, classification, and prognosis [104, 107, 112]. Particularly in light of the recently described pre-malignant conditions CHIP, ICUS, and CCUS, there is a need to further investigate the molecular landscape in MDS. Due to new technological advances, that is, whole exome sequencing (WES), whole genome sequencing (WGS), and whole transcriptome sequencing (WTS), it is now possible to gain a genome-wide molecular insight that not only tracks the mutational status but also measures gene expression and detects cytogenetic aberrations. In MDS, the implementation of gene mutations into the IPSS-M (molecular) represents the next step; this is currently underway driven by efforts of the International Working Group for Prognosis in MDS (IWG-PM). Since the clinical course in MDS is quite heterogeneous, the definition of “best treatment” and goals for outcome would most likely benefit from incorporation of cytogenetic and molecular genetic findings.

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