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REVIEW ARTICLE

Myelodysplastic Syndrome/Myeloproliferative Neoplasm (MDS/MPN) Overlap Syndromes: Molecular Pathogenetic Mechanisms and Their Implications

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Abstract The MDS/MPN overlap syndromes are recently evolved entities that have been quite difficult to define since their discovery. They have overlapping features with other myeloid neoplasms such as MDS and MPN, which further complicates the task of their diagnosis. The unravelling of their molecular pathogenesis by recent diagnostic innovations was of paramount significance in understanding the mechanism of these syndromes. The identification of the major genetic pathways implicated in their pathogenesis not only will help in their diagnosis, but also will enable development of targeted molecular therapy as well as prognostic markers. This review discus the basic molecular aberrations in MDS/MPN overlap syndromes and their possible future implications.

Keywords MDS/MPN · Pathogenesis · Mutations · Signalling pathways · Splicing · Epigenetics

Introduction

Myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndromes are a group of chronic clonal myeloid malignancies in which there are features of both MDS and MPN at the time of presentation [1]. The MDS/MPN overlap syndromes were first described as a separate entity in the 3rd edition of "WHO classification of tumours of Hematopoietic and Lymphoid tissues" with 3 distinct entities namely atypical chronic myeloid leukemia (aCML), chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML) and two provisional entities namely refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) and myelodysplastic syndrome/myeloproliferative neoplasm-unclassified (MDS/MPN-U).

In the 4th edition, however, MDS/MPN-U was made a definite entity while RARS-T remained a provisional entity under MDS/MPN-U. The threshold for thrombocytosis in RARS-T was reduced from $600 \times 10^3/\mu L$ to $450 \times 10^3/\mu L$ along with inclusion of bone marrow criteria in this edition (i.e.) presence of large megakaryocytes resembling those in essential thrombocythaemia (ET). In the revised 4th edition of WHO published in 2017 [1], RARS-T was also made a distinct entity and renamed as myelodysplastic syndrome/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T). The present classification is a concise perspective of four independent panels of laboratory and clinical experts endorsed in 2013–2014 by the MDS foundation.

Classification of MDS/MPN Overlap Syndromes [1]

1. Atypical chronic myeloid leukemia, BCR-ABL negative (aCML)
2. Chronic myelomonocytic leukemia (CMML)
3. Juvenile myelomonocytic leukemia (JMML)
4. Myelodysplastic syndrome/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)

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5. Myelodysplastic syndrome/myeloproliferative neoplasm-Unclassifiable (MDS/MPN-U)

The overlap of MDS and MPN features in these syndromes have been identified at all levels including clinical features, pathogenesis, molecular mutations and treatment options. Because of this overlap, there has been an inherent difficulty in the diagnosis and classification of these neoplasms. Unlike other neoplasms, counts could not be used to classify these neoplasms since there could be either cytopenias or increased counts (due to dysplastic and proliferative features respectively). Morphology is an important tool in their diagnosis, but their subjective nature limits their use to an extent. This problem is further compounded by the fact that the morphology of classical MPNs evolve over time and may show dysplastic features on progression. These impediments necessitated the discovery of more objective ways to diagnose them. This lead to the identification of molecular and cytogenetic abnormalities which drove their pathogenesis. Though disease defining abnormalities have not yet been found, the present knowledge of these aberrations offers better understanding of these neoplasms and a prelude to define these disorders at the molecular level.

Pathogenesis of MDS/MPN Overlap Syndromes

The clinical heterogeneity of these syndromes can be attributed to the multistep pathogenesis which involves the acquiring of various combinations of mutations responsible for both the dysplastic and proliferative features. These mutations can either be driver or passenger mutations. However, none of these mutations are necessary for the development of MDS/MPN.

The origin of these neoplasms may be traced to pluripotent lymphoid-myeloid stem cell or in some cases, a committed myeloid progenitor [1]. Due to the lack of well-defined clinical and molecular markers, there is a paucity of data on the true prevalence of these disorders. There is no prevalence study of these disorders from India as of now.

The subcellular abnormalities in adult onset MDS/MPN (aCML, CMML, MDS/MPN-RS-T, MDS/MPN-U) can be broadly divided into cytogenetic and somatic mutations.

Cytogenetic Abnormalities

Conventional karyotyping and high resolution single nucleotide polymorphism array (SNP-A) identified chromosomal abnormalities in around 70% of patients [2]. They are usually aneuploidies such as trisomy 8, trisomy 9, monosomy 7 and deletions such as del7q, del13q, del20q [3, 4]. Some of them have reciprocal translocations involving tyrosine kinases, however, those involving rearrangement of the genes PDGFRA, PDFRB and FGFR1 are grouped under the WHO category "myeloid and lymphoid neoplasms with eosinophilia". The significance of identifying and classifying these mutations separately is due to the availability of targeted therapy [5–9].

Molecular Abnormalities

A normal cell has a tightly regulated intracellular signalling pathway that helps relay growth signals from the extracellular mileu to the nucleus of the cell where critical response elements on the DNA are triggered leading to suppression or overexpression of factors that result in cell proliferation. These DNA segments may be coding sequences (exons) or non-coding sequences (introns) that influence DNA expression. The DNA is then transcribed to mRNA, which undergoes splicing and translation to form functional proteins. Additional processes such as epigenetic changes and DNA repair responses also affect this process. Any perturbation in this basic pathway by mutagenesis can lead to uncontrolled/disordered cellular proliferation (Fig. 1). Basically these mutations directly or indirectly cause loss of function of tumour suppressor genes or overexpression of oncogenes.

The true incidence of somatic mutations in MDS/MPN overlap syndromes has not been estimated since these syndromes were underdiagnosed till now. The advent of whole genome and whole exome sequencing by next generation sequencing (NGS) has identified more than 40 genes to be mutated in myeloid malignancies [10, 11], some of them playing a central role in disease biology and aid in diagnosis. For example, mutations in JAK2, CBL, TET2 and NRAS cause up-regulation of STAT5 pathway in a cytokine-dependent manner, which is responsible for the unique hypersensitivity of these neoplasms to GM-CSF as measured by hematopoietic colony formation and GM-CSF-dependent phosphorylation of STAT5 [12]. However, none of these genomic variations are specific or unique to MDS/MPN overlap syndromes as they have also been identified in other myeloid neoplasms such as MDS, MPN and AML.

These mutations can be sub-grouped and studied based on their pathway, into four groups;

1. Signal transduction pathway mutations
2. RNA splicing pathway mutations
3. Mutations that affect transcription and DNA damage response
4. Epigenetic mutations

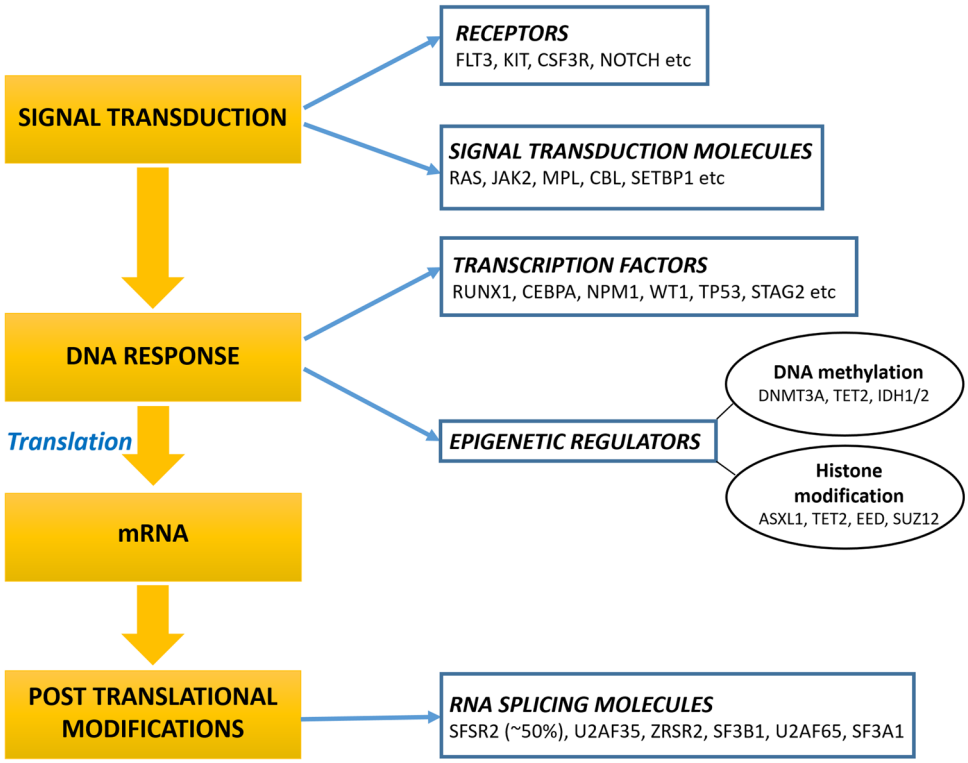

Fig. 1 Cellular signalling pathway and their associated mutations in MDS/MPN overlap syndromes

Signal Transduction Pathway Mutations

These mutations are the most commonly seen in MDS/MPN overlap syndromes. The genes involved may be growth receptors, signal transduction components or negative regulators. These confer proliferative capacities to these neoplasms [13, 14].

RAS

Rat sarcoma (RAS) proteins are a family of small membrane-associated GTPases that regulate several downstream signalling pathways including MAP kinase cascade of serine/threonine kinases. RAS proto-oncogene is mutated mainly in CMML and aCML, with a frequency of about 10–15% [13], opening the avenue for use of PI3K inhibitors. Most of the mutations are seen in KRAS and NRAS in the exons 12, 31 and 61 [15–17].

JAK2 and MPL

Janus kinase 2 (JAK2) mutation is seen in around 10% cases of CMML and 4–8% cases of aCML. It is found in almost 60% cases of MDS/MPN-RS-T, contributing to the thrombocytosis seen in these cases [13]. JAK2 is a non-receptor tyrosine kinase that mediates signalling through EpoR, MPL (TpoR), GCSFR etc. and hence essential for normal hematopoiesis. JAK2V617F mutation results in constitutive activation of the MAPK, PI3K and STAT pathways and therefore altered transcriptional activity [18, 19]. Indirect activation of JAK2 can also happen by the mutations of cMPL gene [20], which is seen in around 5–20% cases of MDS/MPN-RS-T.

CBL

Casitas B Lymphoma (CBL) mutations are seen in about 10–20% cases of CMML, 8% cases of aCML and $>$ 10% cases of MDS/MPN-U [13, 21]. CBL protein has both positive and negative roles in tyrosine kinase signalling. The negative activity resides in the E3 ligase domain which ubiquitinates lysine residues leading to proteosomal degradation of itself as well as other proteins such as STAT. CBL mutations inactivate E3 ligase activity resulting in gain of function and subsequent constitutive signalling.

KIT

KIT mutations are seen in $\sim$ 1–4% cases of CMML and aCML [13, 22] but are more common in cases of systemic mastocytosis associated with a clonal haematological non-mast cell disease [23]. The cKITD816V mutation when found in CMML, presents with systemic mastocytosis with or without eosinophilia [24, 25]. These mutations result in

aberrant activation of several downstream signalling pathways such as MAPK/RAS/ERK, PI3K, SRC and PLC-γ.

FLT3

fms-like tyrosine kinase III (FLT3) is a transmembrane tyrosine kinase. Their mutations are seen in about 5% cases of CMML, 1–3% cases of aCML and ~ 3% cases of MDA/MPN-U. The mutations are either activating or internal tandem duplications. FLT3 fusion mutations found in occasional cases of aCML have shown response to FLT3 inhibitors [26, 27].

CSF3R

Colony stimulating factor 3 receptor (CSF3R) with its ligand G-CSF mediates the normal differentiation and maturation of myeloid progenitors. Previously, there was overlap in the diagnosis of CNL and aCML morphologically, leading to overestimation of cKIT mutations in aCML. However, recent studies have concluded that the true incidence of these mutations are much lesser in MDS/MPN [28, 29].

There are two types of mutations in CSF3R with therapeutic implications. The truncation mutations due to frameshifts respond to SRC kinase inhibitors such as dasatinib whereas point mutations in the extracellular domain (membrane proximal mutations) strongly activate the JAK-STAT pathway, hence respond to ruxolitinib [30].

SETBP1

SET binding protein 1 (SETBP1) mutations are most commonly seen in aCML (25%) followed by CMML (6–15%), MDS/MPN-U (10%) and occasional cases of JMML [31]. SETBP1 functions to stabilize SET, which in turn is a negative regulator of the tumour suppressor Protein phosphatase 2A (PP2A). Gain of function mutations in SETBP1 results in increased SET protein which reduces the activity of PP2A leading to cellular proliferation [32].

Others

The other genes mutated in the group of signalling pathway are NOTCH, PTPN11 and NF1. While loss of mutations in the NOTCH pathway occur rarely in MDS/MPN, mutations of PTPN11 and NF1 are frequently seen in JMML and shall be discussed separately.

RNA Splicing Pathway Mutations

Splicing is the process by which pre-mRNA is converted into mRNA by excision of introns and joining of coding exons. This is done by a mutation targeted machinery called the spliceosome. The spliceosome is composed of five small nuclear ribonucleoproteins and several associated proteins [33]. Mutations in the spliceosome components are mutually exclusive and alter the specificity of splicing causing changes at protein level. These mutations are almost exclusively seen in CMML, in about 60% cases. The genes most commonly implicated in order of frequency are SFSR2 (~ 50%), U2AF35, ZRSR2, SF3B1, U2AF65, SF3A1 etc. [13].

One of these mutations deserves special mention. The Splicing factor 3B1 (SF3B1) gene is mutated in almost 80% cases of MDS/MPN-RS-T [13]. The presence of this mutation correlates with the morphological presence of ring sideroblasts, characterized by abnormal coarse iron deposition in the mitochondria of erythroblasts. This is believed to be due to the abnormal splicing of the gene SLC25A37, which functions as an importer of Fe^{2+} into the mitochondria [34].

Mutations that Affect Transcription and DNA Damage Response

The genes most commonly mutated in this group include RUNX1, CEBPA, NPM1, WT1, TP53 and cohesion complex genes. RUNX1 gene mutations are seen in around 15–37% cases of CMML and are rare in other types of MDS/MPN [13]. The RUNX1 gene is more commonly involved in leukemic translocations and hence, MDS/MPN cases with these mutations are associated with higher risk of leukemic transformation [35]. CEBPA (CCAAT/enhancer-binding protein-alpha) mutations have been seen in 4–20% cases of CMML [35]. They are usually acquired mutations but sometimes can also be inherited. TP53 mutations are associated with a higher risk of AML transformation and hence their presence connotes a poor prognosis. Mutations of the cohesin complex genes such as SMC1, SMC3, RAD21 and STAG2 are seen in around 10% cases of CMML. These proteins are multimeric and involved in sister chromatid separation during cell division and DNA repair [36].

Epigenetic Mutations

Epigenetics refers to inherited changes in phenotype due to chromosomal changes not involving the DNA sequence. The two important classes of protein in epigenetics include those involved in DNA methylation and histone modification. These genes are commonly mutated in MDS/MPN.

DNA Methylation

The most common genes mutated in this group include DNMT3A, TET2 and IDH1/2. DNA methyl transferase 3A (DNMT3A), as the name suggests is a methyl transferase enzyme which adds methyl group to 5′ cytosine in CpG dinucleotides to form 5-methylcytosine. This methylation results in global gene silencing. TET2 (Ten-Eleven translocations 2) mediates the conversion of 5-methylcytosine to 5-hydroxymethylcytosine causing functional demethylation. This reaction requires α-ketoglutarate, which is produced from isocitrate by IDH1/2 (Isocitrate dehydrogenase 1/2) through the Kreb's cycle [37].

Gain-of-function mutations in IDH1/2 produce 2-hydroxyglutarate rather than α-ketoglutarate, which inhibits TET2. The loss of function of TET2 causes global promoter hypermethylation. Mutations in IDH1/2 and TET2 are mutually exclusive [37]. TET2 mutations are quite common in MDS/MPN and are seen in 40–60% cases of CMML and 30% cases of aCML. DNMT3A and IDH1/2 mutations are less common (< 10%); however DNMT3A mutations are seen in 17% cases of MDS/MPN-RS-T [13]. The levels of 2-hydroxyglutarate as a biomarker for the mutational status of IDH1/2 and TET2 have been explored in many studies, especially in MDS.

Histone Modifications

Histones are DNA associated proteins that help in organizing the chromatin into nucleosomes. These histones can influence genetic expression by undergoing modifications such as methylation, acetylation, phosphorylation etc. Any perturbance in these modification processes can lead to changes at the protein level.

Histone H3 lysine 27 methyltransferase (H3K27) is one such enzyme. It has an active catalytic subunit called Polycomb Repressive Complex 2 (PRC2) which is encoded by the Enhancer of Zeste Homologue 2 (EZH2) gene. Loss of function mutations of the EZH2 gene are seen in around 10% of MDS/MPN cases and are associated with poor prognosis [38]. They are early events in mutagenesis. Other genes mutated in this group include EED, SUZ12, UTX etc.

Additional of Sex Combs-like (ASXL1) is a protein that recruits the PRC2 complex to the histones. Loss of function mutations of ASXL1 is a driver event in MDS/MPN since

Table 1 Prevalence of different mutations in aCML, CMML, MDS/MPN-U and MDS/MPN-RS-T

Cellular pathway affected	Gene	Prevalence			
		aCML	CMML	MDS/MPN-U	MDS/MPN-RS-T
Signal transduction	RAS	~ 10%	~ 10%	~ 10% *(NRAS)*	
	JAK2	4–8%	10%		60%
	MPL				~ 20%
	CBL	8%	10–20%	~ 10%	
	KIT	~ 5%	~ 5%		
	FLT3	5%	Rare	Rare	
	CSF3R	~ 10%	Rare	Rare	
	SETBP1	25%	6–15%	10%	
RNA splicing	SF3B1		~ 6%		80%
	SFSR2		~ 40%		Rare
	U2AF35		5–15%		
	ZRSR2		~ 10%		Rare
Transcription and DNA damage response	RUNX1	5–10%	15–37%	> 10%	
	CEBPA	< 1%	4–20%	< 1%	
	NPM1	< 1%	1–6%	< 5%	
	WT1		Rare	Rare	
	TP53	Rare	> 1%	Rare	
Cohesin complex	STAG2		10%		
DNA methylation	DNMT3A	Rare	5–10%	Rare	17%
	TET2	30%	40–60%	30%	25–30%
	IDH1/2	Rare	Rare	5–10%	
Histone modifications	ASXL1	~ 25%	~ 40%		
	EZH2	~ 10%	~ 10%	~ 10%	

they promote myeloid transformation by loss of polycomb repression [39]. They are the most common mutations in CMML, seen in around 40% of cases [13]. Though some have studied ASXL1 to define a prognostically distinct group of CMML, further studies failed to reproduce this association [40]. Table 1 highlights the prevalence of different mutations and their associated different cellular signalling pathways (Fig. 1) in various MDS/MPN overlap syndromes.

Pathogenesis of JMML

JMML is an MDS/MPN overlap syndrome whose molecular pathogenesis has been studied extensively. Hypersignalling of the RAS pathway is fundamental to the development of JMML. This hyperactivity is caused by somatic or germline mutations in NRAS, KRAS, NF1, PTPN11 and CBL genes in around 90% cases of JMML [41, 42]. These mutations are mutually exclusive. Hyperactivation of RAS has been seen in several autosomal dominant developmental disorders with germline mutations of these genes termed as RASopathies, in which there is also an increased risk of JMML. Study of these disorders have helped a great deal in shedding light on the pathogenesis of JMML.

RAS protein is involved in intracellular signal transduction on ligand binding to several receptors, in particular the GM-CSF receptor, thereby mediating cell proliferation. RAS protein in its active configuration is bound to GTP while the inactive form is bound to GDP. Therefore the activity of RAS is regulated by guanosine nucleotide exchange factors and GTPase activating proteins (GAPs), the former activating RAS by exchanging GTP for GDP and the latter inhibiting RAS by causing hydrolysis of GTP.

Neurofibromatosis 1 (NF1) gene is mutated in 10–25% cases of JMML and the risk of JMML is increased by 300- to 500-fold in children with NF1, an autosomal dominant syndrome. NF1 gene encodes neurofibromin, a GAP protein and thereby acts as a tumour suppressor. Two hits are required for NF1 inactivation. In germline cases, the second hit may be caused by deletion or uniparental disomy of the mutant allele [43].

RAS mutations are seen in 25% cases of JMML. Activating point mutations are found in codon 12, 13 and 61 of NRAS and KRAS [44].

Noonan syndrome, a developmental disorder caused by germline mutations in PTPN11 is characterized by higher risk of developing JMML-like myeloproliferative disorder. Somatic mutations of PTPN11 are seen in 35% cases of JMML [45] and hence germline mutations need to be excluded. PTPN11 encodes SHP-2, a non-receptor protein tyrosine phosphatase. Mutations result in loss of basal autoinhibition of this enzyme causing constitutive activation and downstream signalling [46].

CBL mutations are seen in 17% cases of JMML. As already described, CBL is a ubiquitin ligase which causes proteosomal degradation of several tyrosine kinases including RAS. The mutations cause loss of function of CBL and may be somatic or germline. A characteristic feature of JMML caused by germline mutations of CBL-gene is the high rate of spontaneous resolution, advocating expectant management in such cases [47].

The discovery of the strong association and causative role of these genetic mutations in JMML has led to the revision of the diagnostic criteria. Along with clinical and haematological criteria, one of the genetic criteria is required for diagnosis. These genetic criteria include mutations of PTPN11, KRAS, NRAS, NF1 and CBL [1]. Targeted therapy is being developed against these molecules, highlighting their pivotal role in JMML pathogenesis. Table 2 shows the characteristic features of different mutations and their mechanisms (Fig. 2) causing RAS hyperactivity in JMML.

Table 2 Characteristic features of different mutations causing RAS hyperactivity in JMML

Gene	Frequency (%)	Protein	Type of mutation	Distinct clinical features
PTPN-11	35	SHP-2	Somatic, gain-of-function	Aggressive course
KRAS/ NRAS	25	KRAS/NRAS	Somatic, gain-of-function	NRAS mutated JMML shows normal HbF, monosomy 7 is common in KRAS mutated JMML
NF-1	10–25	Neurofibromin	Somatic or germline, loss-of-function	Thrombocytosis, increased blasts, onset after the age of 5 years
CBL	17	CBL	Somatic or germline, loss-of-function	Vasculitis, neurologic symptoms, NS-like features, spontaneous regression of JMML

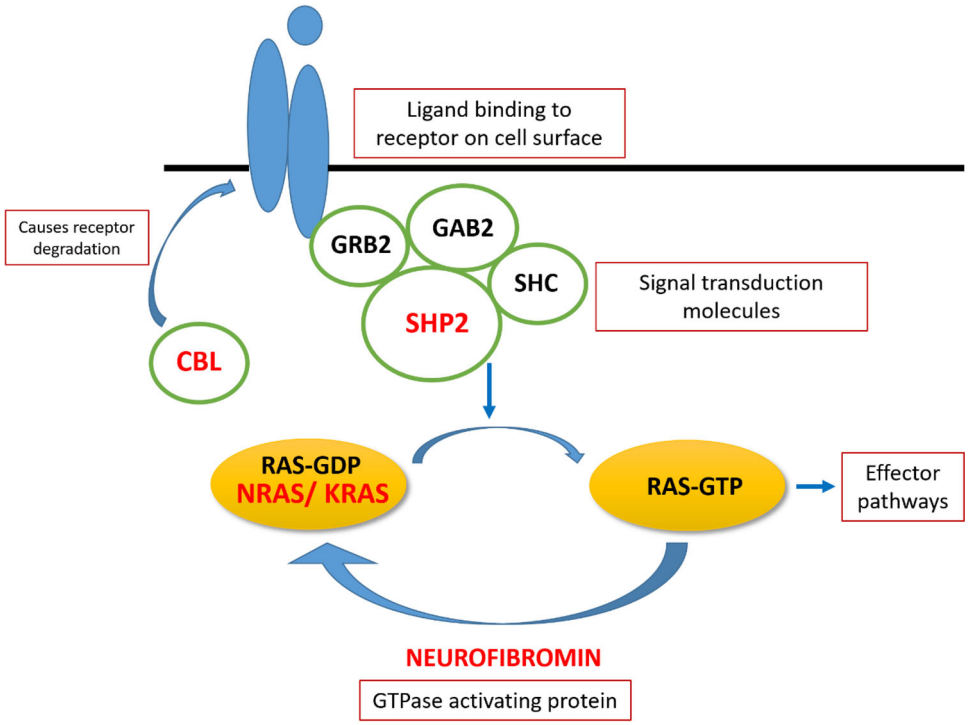

Fig. 2 Mechanism of RAS hyperactivity in JMML (The genes mutated are highlighted in red) (color figure online)

Table 3 Changes in the present WHO classification of MDS/MPN overlap syndromes with respect to cytogenetic and molecular aberrations

Syndrome	Molecular/cytogenetic criteria included in WHO 2017 classification
CMML	Mutations in certain genes such as TET2, SRSF2, ASXL1 and SETBP1 support the diagnosis of CMML in appropriate clinical context. However, these mutations need to be interpreted with caution, since they can be present in other conditions
aCML	A diagnosis of aCML is supported by the presence of SETBP1 and ETNK1 mutations. The presence of CSF3R mutation should prompt careful exclusion of chronic neutrophilic leukemia due to its morphological similarity with aCML
JMML	One genetic criteria is essential for the diagnosis of JMML, which includes: Somatic mutation in PTPN11, KRAS or NRAS Clinical diagnosis of neurofibromatosis type 1 or NF1 mutation Germline CBL mutation and loss of heterozygosity of CBL In the absence of genetic criteria, diagnosis can be made in the presence of monosomy 7 or $\geq$ 2 of the following: Increased HbF for age Circulating myeloid or erythroid precursors In vitro hypersensitivity to GM-CSF Hyperphosphorylation of STAT5
MDS/MPN-RS-T	Even in the presence of SF3B1 mutation, a cut off of $\geq$ 15% ring sideroblasts is essential for this diagnosis. The presence of SF3B1 mutation along with JAK2V617F, CALR or MPL mutation supports this diagnosis

Conclusion

MDS/MPN overlap syndromes are a heterogenous group of disorders that posed difficulty in diagnosis in the yesteryears. The recent availability of molecular diagnostics such as next generation sequencing has helped to study the subcellular events that cause MDS/MPN. The understanding of the pathogenesis of these disorders provides aid to their prevalence, diagnosis and surveillance. The present WHO 2017 classification has brought about certain changes that holds testimonial to the importance of these molecular and cytogenetic aberrations (Table 3) [1]. In the future, genetic signatures will be developed that help in their diagnostic and prognostic subgrouping. Development of targeted therapy in future can help to tailor therapy and increased survival.

References

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Thiele J (2017) WHO classification of tumours of haematopoietic and lymphoid tissue, Revised 4th edn. IARC, Lyon, p p10
2. Tiu RV, Gondek LP, O'Keefe CL et al (2011) Prognostic impact of SNP array karyotypingin myelodysplastic syndromes and related myeloid malignancies. Blood 117(17):4552–4560
3. Cazzola M, Malcovati L, Invernizzi R (2011) Myelodysplastic/myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2011:264–272
4. Delhommeau F, Pisani DF, James C et al (2006) Oncogenic mechanisms in myeloproliferative disorders. Cell Mol Life Sci 63(24):2939–2953
5. James C, Ugo V, Le Couédic JP et al (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycthemia vera. Nature 434(7037):1144–1148
6. Cools J, DeAngelo DJ, Gotlib J et al (2003) A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes is a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 348(13):1201–1214
7. Chase A, Bryant C, Score J, Cross NC (2003) Ponatinib as targeted therapy for FGR1 fusions associated with the 8p11 myeloproliferative syndrome. Haematologica 98(1):103–106
8. Lierman E, Selleslag D, Smits S, Bilet J, Vandenberghe P (2012) Ruxolitinib inhibits transforming JAK2 fusion proteins in vitro and induces complete remission in t(8;9)(p22;p24)/PCM1-JAK2-positive chronic eosinophilic leukemia. Blood 120(7):1529–1531
9. Chase A, Bryant C, Score J et al (2013) Ruxolitinib as potential targeted therapy for patients with JAK2 rearrangements. Haematologica 98(3):404–408
10. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G et al (2014) Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 28:241–247
11. Kohlmann A, Grossmann V, Nadarajah N, Haferlach T (2013) Next generation sequencing—feasibility and practicality in haematology. Br J Haematol 160:736–753
12. Wang J, Liu Y, Li Z et al (2010) Endogenous oncogenic NRAS mutation promotes aberrant GM-CSF signaling in granulocytic/monocytic precursors in a murine model of chronic myelomonocyticleukemia. Blood 116(26):5991–6002
13. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M et al (2013) Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol 31:2428–2436
14. Padron E, Painter JS, Kunigal S, Mailloux AW, McGraw K, McDaniel JM et al (2013) GM-CSF-dependent pSTAT5 sensitivity is a feature with therapeutic potential in chronic myelomonocyticleukemia. Blood 121:5068–5077
15. Braun BS, Tuveson DA, Kong N, Le DT, Kogan SC, Rozmus J et al (2004) Somatic activation of oncogenic Kras in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. Proc Natl Acad Sci USA 101:597–602
16. Chan IT, Kutok JL, Williams IR, Cohen S, Kelly L, Shigematsu H et al (2004) Conditional expression of oncogenic K-RAS from its endogenous promoter induces a myeloproliferative disease. J Clin Invest 113:528–538
17. Van Meter ME, Diaz-Flores E, Archard JA, Passegue E, Irish JM, Kotecha N et al (2007) K-RASG12D expression induces hyperproliferation and aberrant signaling in primary hematopoietic stem/progenitor cells. Blood 109:3945–3952
18. Cross NC (2011) Genetic and epigenetic complexity in myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2011:208–214
19. Oh ST, Gotlib J (2010) JAK2 V617F and beyond: role of genetics and aberrant signaling in the pathogenesis of myeloproliferative neoplasms. Expert Rev Hematol 3:323–337
20. Szpurka H, Gondek LP, Mohan SR, Hsi ED, Theil KS, Maciejewski JP (2009) UPD1p indicates the presence of MPL W515L mutation in RARS-T, a mechanism analogous to UPD9p and JAK2 V617F mutation. Leukemia 23:610–614
21. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C et al (2009) Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. Blood 113:6182–6192
22. Itzykson R, Kosmider O, Renneville A, Morabito M, Preudhomme C, Berthon C et al (2013) Clonal architecture of chronic myelomonocyticleukemias. Blood 121:2186–2198
23. Valent P, Arock M, Akin C, Sperr WR, Reiter A, Sotlar K et al (2010) The classification of systemic mastocytosis should include mast cell leukemia (MCL) and systemic mastocytosis with a clonal hematologic non-mast cell lineage disease (SM-AHNMD). Blood 116:850–851
24. Machherndl-Spandl S, Sega W, Bosmuller H, Germing U, Gruber C, Nachtkamp K et al (2014) Prognostic impact of blast cell counts in dysplastic bone marrow disorders (MDS and CMML I) with concomitant fibrosis. Ann Hematol 93:57–64
25. Sotlar K, Marafioti T, Griesser H, Theil J, Aepinus C, Jaussi R et al (2000) Detection of c-kit mutation Asp 816 to Val in microdissected bone marrow infiltrates in a case of systemic mastocytosis associated with chronic myelomonocytic leukaemia. Mol Pathol 53:188–193
26. Grand FH, Iqbal S, Zhang L, Russell NH, Chase A, Cross NC (2007) A constitutively active SPTBN1-FLT3 fusion in atypical chronic myeloid leukemia is sensitive to tyrosine kinase inhibitors and immunotherapy. Exp Hematol 35:1723–1727
27. Walz C, Erben P, Ritter M, Bloor A, Metzgeroth G, Telford N et al (2011) Response of ETV6-FLT3-positive myeloid/lymphoid neoplasm with eosinophilia to inhibitors of FMS-like tyrosine kinase 3. Blood 118:2239–2242
28. Wang SA, Hasserjian RP, Fox PS, Rogers HJ, Geyer JT, Chabot-Richards D et al (2014) Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. Blood 123:2645–2651
29. Pardanani A, Lasho TL, Laborde RR, Elliott M, Hanson CA, Knudson RA et al (2013) CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. Leukemia 27:1870–1873
30. Maxson JE, Gotlib J, Pollyea DA, Fleischman AG, Agarwal A, Eide CA et al (2013) Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med 368:1781–1790
31. Piazza R, Valletta S, Winkelmann N, Redaelli S, Spinelli R, Pirola A et al (2013) Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet 45:18–24
32. Minakuchi M, Kakazu N, Gorrin-Rivas MJ, Abe T, Copeland TD, Ueda K, Adachi Y (2001) Identification and characterization of SEB, a novel protein that binds to the acute undifferentiated leukemia-associated protein SET. Eur J Biochem 268:1340–1351
33. Chen HC, Cheng SC (2012) Functional roles of protein splicing factors. Biosci Rep 32:345–359
34. Visconte V, Avishai N, Mahfouz R, Tabarroki A, Cowen J, Sharghi-Moshtaghin R et al (2014) Distinct iron architecture in SF3B1-mutant myelodysplastic syndrome patients is linked to an SLC25A37 splice variant with a retained intron. Leukemia 29(1):188–195
35. Ernst T, Chase A, Zoi K, Waghorn K, Hidalgo-Curtis C, Score J et al (2010) Transcription factor mutations in myelodysplastic/myeloproliferative neoplasms. Haematologica 95:1473–1480

36. Kon A, Shih LY, Minamino M, Sanada M, Shiraishi Y, Nagata Y et al (2013) Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet 45:1232–1237
37. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A et al (2010) Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 18:553–567
38. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV et al (2010) Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 42:722–726
39. Abdel-Wahab O, Adli M, LaFaveLM Gao J, Hricik T, Shih AH et al (2012) ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell 22:180–193
40. Patnaik MM, Padron E, Laborde RR et al (2013) Mayo prognostic model for WHO-Defined chronic myelomonocytic leukemia-ASXL1 and spliceosome component mutations and outcomes. Leukemia 27(7):1504–1510
41. Niemeyer CM, Kratz CP (2008) Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol 140(6):610–624
42. Loh ML (2011) Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol 152(6):677–687
43. Shannon KM, O'Connell P, Martin GA et al (1994) Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med 330(9):597–601
44. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J et al (1999) RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia 13(1):32–37
45. Tartaglia M, Niemeyer CM, Fragale A et al (2003) Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet 34(2):148–150
46. Loh ML, Vattikuti S, Schubbert S et al (2004) Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood 103(6):2325–2331
47. Pérez B, Mechinaud F, Galambrun C et al (2010) Germline mutations of the CBL gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukaemia. J Med Genet 47(10):686–691