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Advances in the Study of High-Risk Gene Mutations for Primary Myelofibrosis

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Abstract

Primary myelofibrosis is a kind of MPNs due to clonal appreciation of hematopoietic stem cells. With the development of second-generation sequencing, high-risk mutation (HMR) genes such as ASXL1, EZH2, SRSF2, and IDH1/2 have been shown to be associated with disease prognosis and progression, and although allo-HSCT remains the only possible treatment for PMF, with the development of JAK inhibitors, there is an increasing interest in the study of inhibitors of these mutant loci.

Keywords

Primary Myelofibrosis, High-Risk Mutations, Myeloproliferative Neoplasms

1. Introduction

Myeloproliferative neoplasms (MPNs) are a group of myeloid neoplastic disorders caused by the clonal increase of one or more lineages of relatively mature differentiated myeloid cells. The typical of them are Polycythemia vera (PV), Essential thrombocythemia (ET) and Primary myelofibrosis (PMF), (pre-PMF) and fibrosis (overt-PMF) [1]. In recent years, with the development of high-throughput sequencing technology, the mutations in MPN genes have become better understood, including driver and non-driver genes, the former including JAK2, CARL, MPL, while the latter including ASXL1, TET2, SRSF2, DNMT3A, etc. The combination of mutations through driver genes and hematological and morphological abnormalities represent the main diagnostic criteria. And with recent years, studies have shown that high-risk mutations (HMR) including ASXL1, SRSF2, IDH1/2, EZH2, are associated with prognosis and risk of leu-

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kemic transformation in PMF [2] [3].

2. The Physiological Function of HMR and Its Pathogenic Mechanism

1) ASXL1: *Additional sex combs-like* 1 (ASXL1) is one of the most common mutation types [2] [4]. ASXL1 is located on chromosome 20q11 and encodes 1541 amino acids. ASXL1, an epigenetic regulator, was originally identified as an enhancer of trithorax group (TrxG) and polycomb group (PcG) genes to regulate the expression of Hox genes [5] [6]. The PcG gene represses and the TrxG gene activates Hox gene expression, showing that ASXL has both gene repression and activation. BAP1 can synergize with ASXL1/2 for tumor suppressive effects, but the exact mechanism remains to be investigated [7]. Alterations in ASXL1 are mutations and/or deletions. Mutations in ASXL1 often lead to increased apoptosis and mitosis in bone marrow cells and hematopoietic stem cells [8] [9] [10], leading to changes towards MPN/MDS.

2) EZH2: *Enhancer of zeste gene homolog* 2 (EZH2) is located on chromosome 7q35-q36. The vertebrate Polycomb group achieves silencing of target genes through two multiprotein complexes, PRC1 and PRC2, and EZH2, one of the four core components of the PCR2-type complex is a SET structural domain-containing methyltransferase that catalyzes dimethylation and trimethylation of histone H3 at lysine 27 [11]. EZH2 alterations are mainly missense, shift and nonsense mutations. The deletion of EZH2 is often associated with Jak2V617F in MF, which can contribute to the value-added of Jak2V617 HSC and exhibit erythroid suppressive effects [12]. And its overexpression may be associated with carcinogenesis [13].

3) SRSF2: *Serine/arginine-rich splicing factor* 2 (SRSF2) is located on chromosome 17q25.1.SR protein plays an important role in regulating constitutive and selective pre-mRNA splicing, function. The function is mainly achieved through two structural domains: the N-terminal RNA recognition motif (RRM) structural domain involved in sequence-specific RNA binding, and the C-terminal arginine-rich/serine (RS) structural domain interacting with other splicing factors [14] [15]. SRSF2 mutations are most commonly histidine (P95H) substituted, *i.e.*, expressed in the HSC population prompting the inherent hematopoietic cell myeloid bias/proliferation [16]. And this mutation induces a disabling splicing change in EZH2 [17].

4) IDH1/2: *Isocitrate dehydrogenase* 1 is located on chromosome 2q33.3 and IDH2 is located on chromosome 15q26.1. IDH1 is located in the cytosol and peroxisomes, while IDH2 is located in the mitochondria and functions as an enzyme in the tricarboxylic acid cycle that converts isocitrate to α-ketoglutarate in the tricarboxylic acid cycle. Mutations in both can lead to excessive production of 2-hydroxyglutarate (2HG) from α-ketoglutarate, resulting in histone hypermethylation, and abnormal chromatin modifications that prevent specific progenitor cell differentiation [18]. IDH and JAK2 can interact and their combined expression can induce MPN progression [19].

3. Prognostic Assessment System and Risk Stratification of PMF

The IPSS score is the earliest and most widely used assessment system that classifies risk groups by five variables: age > 65 years, HB < 10 g/dl, white blood cell count > 25 × 10^9/L, peripheral blood primitive cells > 1%, and systemic symptoms, 0 (low risk group), 1 (intermediate risk group 1), 2 (intermediate risk group 2), or greater than or equal to 3 (high risk group), which corresponds to a median survival of 135, 95, 48 and 27 months [20]. The IPSS was subsequently adapted and upgraded to DIPSS, DIPSS-PLUS [21] [22], and risk stratification was newly assigned. With the progressive study of non-driver mutations, the MIPSS70 scoring system for transplant-eligible PMF patients aged < 70 years was developed: a score of 1 for the categories of hemoglobin < 100 g/L, peripheral blood primitive cells > 2%, myelofibrosis > grade 2, systemic symptoms, absence of CARL1 mutation, HMR, and a white blood cell count < 25 × 10^9/L, and platelet count < 100 × 10^9/L, and the presence of ≥ 2 HMR mutations were scored as 2. The risk stratification was also divided into low risk group (0 - 1), intermediate risk group (2 - 4), and high risk group (≥5) with corresponding median survival of 27.7, 6.3, and 2.3 years [23]. u2AF1Q157 as an additional high molecular risk (HMR) mutation and a new sex- and severity-adjusted hemoglobin threshold corrected to MIPSS70+2.0 [24]. While GIPSS is based solely on mutation and karyotype.

4. Impact of HMR Mutations on the Prognosis of PMF

The most common mutated gene corresponding to HMR was AXSL1, and in a retrospective study of 879 PMF patients, the frequencies of mutations in non-driver genes were ASXL1 (21.7%), TET2 (9.7%), SRSF2 (8.5%), DNMT3A (5.7%), EZH2 (5.1%), CBL (4.4%) and IDH1/2 (2.6%) [2], which is consistent with the results of several other studies [4] [25], where ASXL1 mutations were more likely to occur in the presence of normal karyotypes and did not differ between favorable and unfavorable cytogenetic categories. EZH2, SRSF2 and IDH1/2 mutation frequencies were similar in patients with normal and abnormal karyotypes. The ASXL1 mutation was associated with leukocytosis, systemic symptoms, and >1% peripheral primitive cells; the SRSF2 mutation was associated with older age and >1% peripheral primitive cells; and the EZH2 mutation was associated with >1% peripheral primitive cells. The frequency of >1 HMR mutation was increased in the high-risk group in several studies [25] [26], while the 5-year survival (OS) was significantly lower in the group of patients with HMR mutations compared to the group without HMR mutations, 47.5% and 85%, respectively [25]. In a previous long-term prognostic study of 1282 PMF patients, early mortality in PMF was found to be associated with genetic risk factors, while survival beyond 20 years could be predicted by clinical variables including age, sex, blood count, and symptoms [27]. Since EZH2, ASXL1 and SRSF2 mutations predict overall survival, and ASXL1, SRSF2 and IDH1 or IDH2 are inde-

pendent predictors of leukemic transformation [2] [28], we suggest that these mutations (ASXL1, EZH2, SRSF2 and IDH1/2) have a negative outcome on the disease. And the presence of HMR mutations accelerates the transformation of pre-MF towards overt-PMF and AML [3].

5. Progress in the Treatment of PMF

In the treatment of anemia, androgens, glucocorticoids or thalidomide/lenalidomide, prednisone, and danazol are often used, while EPO is commonly used in patients with EPO <100 U/L [29]. In the treatment of splenomegaly, the first-line drug of choice for MF-related splenomegaly remains hydroxyurea, which effectively halves the size of the spleen in approximately 40% of patients [29], and the response of the spleen to hydroxyurea lasts for an average of one year, while bone marrow suppression and painful skin mucosal ulcers are its most common side effects. Irradiation of the splenic region provides short-term relief of the fullness caused by hepatomegaly and splenomegaly, and its main side effect is hematocrit. Interferon α and γ are more effective in myelofibrosis with thrombocytosis. Since JAK2V617, MPL and CALR mutations are driving abnormalities through activation of JAK/STAT signaling, this led to the development of JAK inhibitors. In contrast, ruxolitinib was the first drug of this class to be approved for myelofibrosis, effectively reducing splenomegaly and alleviating systemic symptoms [30]. Ruxolitinib does not have antitumor activity and has not been shown to reverse myelofibrosis or induce cytogenetic or molecular remission. Instead, its mechanism of action is based on its non-specific ability to inhibit inflammatory cytokines. Thus most patients with myelofibrosis taking ruxolitinib become resistant to treatment as symptoms and splenomegaly, hematocrit worsen or progress to an acute phase, with high discontinuation rates and <50% of patients with efficacy maintaining efficacy over 5 years [31]. Many drugs are still under investigation, such as the telomerase inhibitor Imetelstat, a 13-polymer lipid-coupled oligonucleotide that has been tested in MF and ET, with complete or partial responses observed in seven patients whose responses were associated with the presence of JAK2V617F, SF3B1 or U2AF1 mutations and the deletion of the ASXL1 mutation [32]. There are still a number of drugs still being investigated alone or in combination with ruxolitinib, including IDH1/2 antagonists (ivosidenib and enasidenib), CD123 (IL3RA)-directed cytotoxins (IL3 fused with diphtheria toxin) (e.g., tagraxofusp (SL-401), BET inhibitors (e.g., CPI-0610), BCL-2/BCL-X inhibitors (e.g. navitoclax, venetoclax), LSD1 inhibitors (e.g. bomedemstat), PI3/AKT inhibitors (e.g. buparlisib), etc. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only possible treatment for PMF, but has a considerable treatment-related mortality and complication rate. Several studies have shown a survival rate of 40% to 60% at 2 - 5 years after allogeneic transplantation [33] [34]. MAC is suitable for young adults because of the lower recurrence rate and the overall improved Relapse-FreeSurvival (GRFS) advantage. In contrast, reduced-intensity conditioning (RIC) allo-SCT

has a clear survival advantage in older, healthier MF allograft patients and needs to be optimized to reduce recurrence and non-disabling rates [35]. Indications for splenectomy include painful compression or splenic infarction due to splenomegaly, symptomatic portal hypertension, severe thrombocytopenia, and uncontrolled hemolysis [29]. However, splenectomy can result in rapid liver enlargement, and some studies have shown that splenectomy before AHSCT has beneficial effects [36].

6. Outlook

The discovery of JAK2, CALR, and MPL driver mutations has elucidated the genetic basis of most MPNs, and the use of high-throughput NGS technology has further expanded the understanding of these diseases. HMR mutations, among others, can improve diagnosis, stratify risk assessment, optimize clinical decision making in patients eligible for hematopoietic cell transplantation (HCT), and monitor response to therapy. While allo-HSCT may still be the only cure for PMF, the development of JAK inhibitors offers new hope for patients with MPN and has inspired research into other HMR mutation inhibitors.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Arber, D.A., Orazi, A., Hasserjian, R., *et al.* (2016) The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood*, **127**, 2391-2405. https://doi.org/10.1182/blood-2016-03-643544

[2] Vannucchi, A.M., Lasho, T.L., Guglielmelli, P., *et al.* (2013) Mutations and Prognosis in Primary Myelofibrosis. *Leukemia*, **27**, 1861-1869.

[3] Carobbio, A., Guglielmelli, P., Rumi, E., *et al.* (2020) A Multistate Model of Survival Prediction and Event Monitoring in Prefibrotic Myelofibrosis. *Blood Cancer Journal*, **10**, Article No. 100. https://doi.org/10.1038/s41408-020-00368-1

[4] Tefferi, A., Lasho, T.L., Finke, C.M., *et al.* (2016) Targeted Deep Sequencing in Primary Myelofibrosis. *Blood Advances*, **1**, 105-111. https://doi.org/10.1182/bloodadvances.2016000208

[5] Sinclair, D.A., Mine, T.A., Hodgson, J.W., *et al.* (1998) The Additional Sex Combs Gene of Drosophila Encodes a Chromatin Protein that Binds to Shared and Unique Polycomb Group Sites on Polytene Chromosomes. *Development*, **125**, 1207-1216. https://doi.org/10.1242/dev.125.7.1207

[6] Milne, T.A., Sinclair, D.A. and Brock, H.W. (1999) The Additional Sex Combs Gene of Drosophila Is Required for Activation and Repression of Homeotic Loci, and Interacts Specifically with Polycomb and Super Sex Combs. *Molecular Genetics and Genomics*, **261**, 753-761. https://doi.org/10.1007/s004380050018

[7] Micol, J.B. and Abdel-Wahab, O. (2016) The Role of Additional Sex Combs-Like Proteins in Cancer. *Cold Spring Harbor Perspectives in Medicine*, **6**, a026526. https://doi.org/10.1101/cshperspect.a026526

[8] Wang, J., Li, Z., He, Y., *et al.* (2014) Loss of Asxl1 Leads to Myelodysplastic Syndrome-Like Disease in Mice. *Blood*, **123**, 541-553.

https://doi.org/10.1182/blood-2013-05-500272

[9] Abdel-Wahab, O., Gao, J., Adli, M., *et al.* (2013) Deletion of Asxl1 Results in Myelodysplasia and Severe Developmental Defects *in Vivo*. *Journal of Experimental Medicine*, **210**, 2641-2659.

[10] Zhang, P., Chen, Z., Li, R., *et al.* (2018) Loss of ASXL1 in the Bone Marrow Niche Dysregulates Hematopoietic Stem and Progenitor Cell Fates. *Cell Discovery*, **4**, Article No. 4. https://doi.org/10.1038/s41421-017-0004-z

[11] Schuettengruber, B. and Cavalli, G. (2009) Recruitment of Polycomb Group Complexes and Their Role in the Dynamic Regulation of Cell Fate Choice. *Development*, **136**, 3531-3542. https://doi.org/10.1242/dev.033902

[12] Yang, Y., Akada, H., Nath, D., Hutchison, R.E. and Mohi, G. (2016) Loss of Ezh2 Cooperates with Jak2V617F in the Development of Myelofibrosis in a Mouse Model of Myeloproliferative Neoplasm. *Blood*, **127**, 3410-3423. https://doi.org/10.1182/blood-2015-11-679431

[13] Simon, J.A. and Lange, C.A. (2008) Roles of the EZH2 Histone Methyltransferase in Cancer Epigenetics. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **647**, 21-29. https://doi.org/10.1016/j.mrfmmm.2008.07.010

[14] Long, J.C. and Caceres, J.F. (2009) The SR Protein Family of Splicing Factors: Master Regulators of Gene Expression. *Biochemical Journal*, **417**, 15-27. https://doi.org/10.1042/BJ20081501

[15] Zhang, J., Lieu, Y.K., Ali, A.M., *et al.* (2015) Disease-Associated Mutation in SRSF2 Misregulates Splicing by Altering RNA-Binding Affinities. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, E4726-E4734. https://doi.org/10.1073/pnas.1514105112

[16] Smeets, M.F., Tan, S.Y., Xu, J.J., *et al.* (2018) $Srsf2^{P95H}$ Initiates Myeloid Bias and Myelodysplastic/Myeloproliferative Syndrome from Hemopoietic Stem Cells. *Blood*, **132**, 608-621. https://doi.org/10.1182/blood-2018-04-845602

[17] Kim, E., Ilagan, J.O., Liang, Y., *et al.* (2015) SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. *Cancer Cell*, **27**, 617-630. https://doi.org/10.1016/j.ccell.2015.04.006

[18] Kurt, H., Bueso-Ramos, C.E., Khoury, J.D., *et al.* (2018) Characterization of IDH1 p.R132H Mutant Clones Using Mutation-Specific Antibody in Myeloid Neoplasms. *The American Journal of Surgical Pathology*, **42**, 569-577. https://doi.org/10.1097/PAS.0000000000000970

[19] McKenney, A.S., Lau, A.N., Somasundara, A.V.H., *et al.* (2018) JAK2/IDH-Mutant-Driven Myeloproliferative Neoplasm Is Sensitive to Combined Targeted Inhibition. *Journal of Clinical Investigation*, **128**, 789-804. https://doi.org/10.1172/JCI94516

[20] Cervantes, F., Dupriez, B., Pereira, A., *et al.* (2009) New Prognostic Scoring System for Primary Myelofibrosis Based on a Study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*, **113**, 2895-2901. https://doi.org/10.1182/blood-2008-07-170449

[21] Passamonti, F., Cervantes, F., Vannucchi, A.M., *et al.* (2010) A Dynamic Prognostic Model to Predict Survival in Primary Myelofibrosis: A Study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*, **115**, 1703-1708. https://doi.org/10.1182/blood-2009-09-245837

[22] Gangat, N., Caramazza, D., Vaidya, R., *et al.* (2011) DIPSS Plus: A Refined Dynamic International Prognostic Scoring System for Primary Myelofibrosis that Incorpo-

rates Prognostic Information from Karyotype, Platelet Count, and Transfusion Status. *Journal of Clinical Oncology*, **29**, 392-397. https://doi.org/10.1200/JCO.2010.32.2446

[23] Guglielmelli, P., Lasho, T.L., Rotunno, G., Mudireddy, M., *et al.* (2018) MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients with Primary Myelofibrosis. *Journal of Clinical Oncology*, **36**, 310-318.

[24] Tefferi, A., Guglielmelli, P., Lasho, T.L., *et al.* (2018) MIPSS70+ Version 2.0: Mutation and Karyotype-Enhanced International Prognostic Scoring System for Primary Myelofibrosis. *Journal of Clinical Oncology*, **36**, 1769-1770. https://doi.org/10.1200/JCO.2018.78.9867

[25] Morishita, S., Ochiai, T., Misawa, K., *et al.* (2021) Clinical Impacts of the Mutational Spectrum in Japanese Patients with Primary Myelofibrosis. *International Journal of Hematology*, **113**, 500-507. https://doi.org/10.1007/s12185-020-03054-x

[26] Rontauroli, S., Castellano, S., Guglielmelli, P., *et al.* (2021) Gene Expression Profile Correlates with Molecular and Clinical Features in Patients with Myelofibrosis. *Blood Advances*, **5**, 1452-1462. https://doi.org/10.1182/bloodadvances.2020003614

[27] Penna, D., Lasho, T.L., Finke, C.M., *et al.* (2019) 20+ Years and Alive with Primary Myelofibrosis: Phenotypic Signature of Very Long-Lived Patients. *American Journal of Hematology*, **94**, 286-290. https://doi.org/10.1002/ajh.25351

[28] Shahin, O.A., Chifotides, H.T., Bose, P., Masarova, L. and Verstovsek, S. (2021) Accelerated Phase of Myeloproliferative Neoplasms. *Acta Haematologica*, **144**, 484-499. https://doi.org/10.1159/000512929

[29] Leukemia and Lymphoma Group, Chinese Society of Hematology and Chinese Medical Association (2019) Chinese Guideline on the Diagnosis and Treatment of Primary Myelofibrosis. *Chinese Journal of Hematology*, **40**, 1-7.

[30] Harrison, C., Kiladjian, J.J., Al-Ali, H.K., *et al.* (2012) JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. *The New England Journal of Medicine*, **366**, 787-798. https://doi.org/10.1056/NEJMoa1110556

[31] Harrison, C.N., Vannucchi, A.M., Kiladjian, J.J., *et al.* (2017) Long-Term Findings from COMFORT-II, a Phase 3 Study of Ruxolitinib vs Best Available Therapy for Myelofibrosis. *Leukemia*, **31**, Article No. 775. https://doi.org/10.1038/leu.2016.323

[32] Tefferi, A., Lasho, T.L., Begna, K.H., *et al.* (2015) A Pilot Study of the Telomerase Inhibitor Imetelstat for Myelofibrosis. *The New England Journal of Medicine*, **373**, 908-919. https://doi.org/10.1056/NEJMoa1310523

[33] Ballen, K.K., Shrestha, S., Sobocinski, K.A., *et al.* (2010) Outcome of Transplantation for Myelofibrosis. *Biology of Blood and Marrow Transplantation*, **16**, 358-367.

[34] Kerbauy, D.M., Gooley, T.A., Sale, G.E., *et al.* (2007) Hematopoietic Cell Transplantation as Curative Therapy for Idiopathic Myelofibrosis, Advanced Polycythemia Vera, and Essential Thrombocythemia. *Biology of Blood and Marrow Transplantation*, **13**, 355-365. https://doi.org/10.1016/j.bbmt.2006.11.004

[35] McLornan, D., Szydlo, R., Koster, L., *et al.* (2019) Myeloablative and Reduced-Intensity Conditioned Allogeneic Hematopoietic Stem Cell Transplantation in Myelofibrosis: A Retrospective Study by the Chronic Malignancies Working Party of the European Society for Blood and Marrow Transplantation. *Biology of Blood and Marrow Transplantation*, **25**, 2167-2171. https://doi.org/10.1016/j.bbmt.2019.06.034

[36] Bossard, J.B., Beuscart, J.B., Robin, M., *et al.* (2021) Splenectomy before Allogeneic Hematopoietic Cell Transplantation for Myelofibrosis: A French Nationwide Study. *American Journal of Hematology*, **96**, 80-88. https://doi.org/10.1002/ajh.26054