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RNA 剪切复合物突变在骨髓增生异常综合征中的致病机制研究

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摘要 骨髓增生异常综合征(myelodysplastic syndrome, MDS)为中老年人群相对高发的恶性血液系统肿瘤, 治疗难度大且目前无有效的靶向药物。RNA 剪切复合物突变是 MDS 中的高频突变, 探讨其致病机制不仅有助于认识疾病的发生发展规律, 而且可为疾病的精准治疗以及研发靶向 MDS 的药物提供重要的科学依据。该文拟对 RNA 剪切复合物突变在 MDS 中的致病机制、对预后的影响以及其临床转化进行综述, 旨在为 MDS 患者的个体化诊疗提供理论依据。

关键词 骨髓增生异常综合征; RNA 剪切复合物; *SRSF2*; *SF3B1*; *U2AF1*; *ZRSR2*

Study on the Pathogenesis of RNA Splicing Complex Mutation in Myelodysplastic Syndrome

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Abstract MDS (myelodysplastic syndrome) is a hematologic malignancy with relatively high incidence in aged people, which has few effective targeted drugs and is difficult to cure unless hematopoietic stem cell transplantation. RNA splicing complex mutation is a high-frequency mutation identified in MDS patients in recent years. Investigating the pathogenic mechanisms of the mutations of RNA splicing complex not only enhances the under-

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standing of the pathogenesis and progression of MDS, but also provides a critical scientific foundation for developing possible targeted drugs. This study intends to review the pathogenesis, translational research and prognostic value of RNA splicing complex mutation in MDS in order to provide theoretical basis for individualized diagnosis and treatment of patients with MDS.

Keywords myelodysplastic syndrome; RNA splicing complex; *SRSF2*; *SF3B1*; *U2AF1*; *ZRSR2*

骨髓增生异常综合征(myelodysplastic syndrome, MDS)是一种恶性克隆增殖的异质性疾病,以无效造血以及高风险向急性髓系白血病(acute myeloid leukemia, AML)转化为特征^[1-4]。2022年修订的第五版世界卫生组织(World Health Organization, WHO)造血淋巴肿瘤分类:髓系、组织细胞/树突状细胞肿瘤中用“骨髓增生异常肿瘤”代替了“骨髓增生异常综合征”,并且WHO根据疾病的组织特征和遗传学异常重新进行了分类,进而突出了疾病的肿瘤属性^[5]。监测、流行病学和结果数据库(Surveillance, Epidemiology, and End Results, SEER)报道了2001年MDS的年龄校准的年发病率为3.28/100 000,2010年MDS的年龄校准的年发病率为5.6/100 000^[6-7]。MDS发病率在逐年增加,其高发于老年群体中,同时在接受过化疗和/或放疗的癌症幸存者中发病率也较高。MDS的治疗包括支持治疗、免疫调节治疗、免疫抑制治疗、去甲基化药物治疗、化疗、造血干细胞移植等。但是MDS患者的自然病程和预后差异较大,不同预后分层患者的治疗目标不同,对于低危组MDS患者的治疗目标是改善贫血、提高生活品质,对于高危组MDS患者的治疗目标则为延缓疾病进展、延长生存期。目前MDS患者缺乏特异性靶向治疗方法,因此进一步探索MDS的发病机制以及揭示骨髓微环境的变化规律有助于开发新的MDS治疗靶点,进而提高MDS的生活质量和延长患者的生存期。

MDS的主要特征是重现性基因异常、造血细胞发育异常和无效造血导致的多系血细胞减少。基因突变发生在MDS发生发展的各个阶段,80%~90%的MDS患者存在40种常见突变基因中的至少一个突变^[8],最常见的突变涉及表观遗传调控基因*DNMT3A*、*TET2*、*IDH1*和*IDH2*,染色质修饰基因*EZH2*和*ASXL1*,转录调控基因*ETV6*、*RUNX1*和*BCOR*,黏连蛋白复合体组分基因*STAG2*、*CTCF*和*SMC1A*,DNA修复基因*TP53*,RNA剪切复合物相关基因*SF3B1*、*U2AF1*、*SRSF2*和*ZRSR2*,信号传递相关基因*JAK1*、*KRAS*和*CBL*基因^[8-11](表1)。

RNA剪切复合物相关基因(*SRSF2*、*SF3B1*、*U2AF1*和*ZRSR2*)的突变在MDS中很常见,这些突变可能是导致MDS发生造血细胞减少和/或无效造血的原因(图1)^[10]。*SRSF2*突变导致其与RNA基序识别的特异性和亲和力发生改变进而降低RNA剪接效率,导致细胞内RNA广泛的错误剪接,最终错误剪接的RNA被翻译成为功能失调的蛋白质或被无义介导衰变(nonsense-mediated mRNA decay, NMD)降解^[12-13]。*SF3B1*突变在环形铁粒幼细胞(ring sideroblast, RS)伴红系发育不良的人群中较常见,*SF3B1*突变的MDS患者在WHO分类中作为一个独立的亚型,这些患者对罗特西普和来那度胺治疗有较好的反应^[14-16]。在MDS中*U2AF1*突变与不良预后相关^[17-19],其突变位点通常在锌指结构域内的氨基酸S34和Q157上,且*U2AF1*突变可导致3'剪接位点的选择偏倚^[18]。*U2AF1*突变导致自噬基因*ATG7*错误剪接从而抑制自噬导致线粒体功能障碍和基因组不稳定,这些影响使细胞易于发生额外的突变^[20]。

剪接体是由5种小核糖核蛋白复合物(U1、U2、U4、U5、U6)和多种非小核糖核蛋白组成的动态的大分子核糖核蛋白复合物,通过有效识别mRNA前体中的外显子和内含子连接位点,进而协调剪接体分子进行一系列组装、激活、催化和解离反应,最终将内含子去除并将外显子连接产生成熟的mRNA^[21]。在这一生理过程中,如果剪接体复合物基因如*SRSF2*、*SF3B1*、*U2AF1*或*ZRSR2*发生突变则会导致剪接体结构和功能异常,使细胞产生核酸序列异常的mRNA进而翻译出结构及功能异常的蛋白质。因此,本文拟详细对MDS中剪接因子突变的机制以及其对免疫微环境和预后的影响进行综述。

1 *SRSF2*基因突变

*SRSF2*为RNA剪切复合物的核心分子,其蛋白结构包括RNA识别基序(RNA recognition motif, RRM)和丝氨酸精氨酸富集结构域(serine-arginine rich domain, SR domain)。RNA识别结构域的功能

表1 骨髓增生异常综合征患者常见基因突变频率、预后以及潜在的靶向药物(根据参考文献[4]修改)

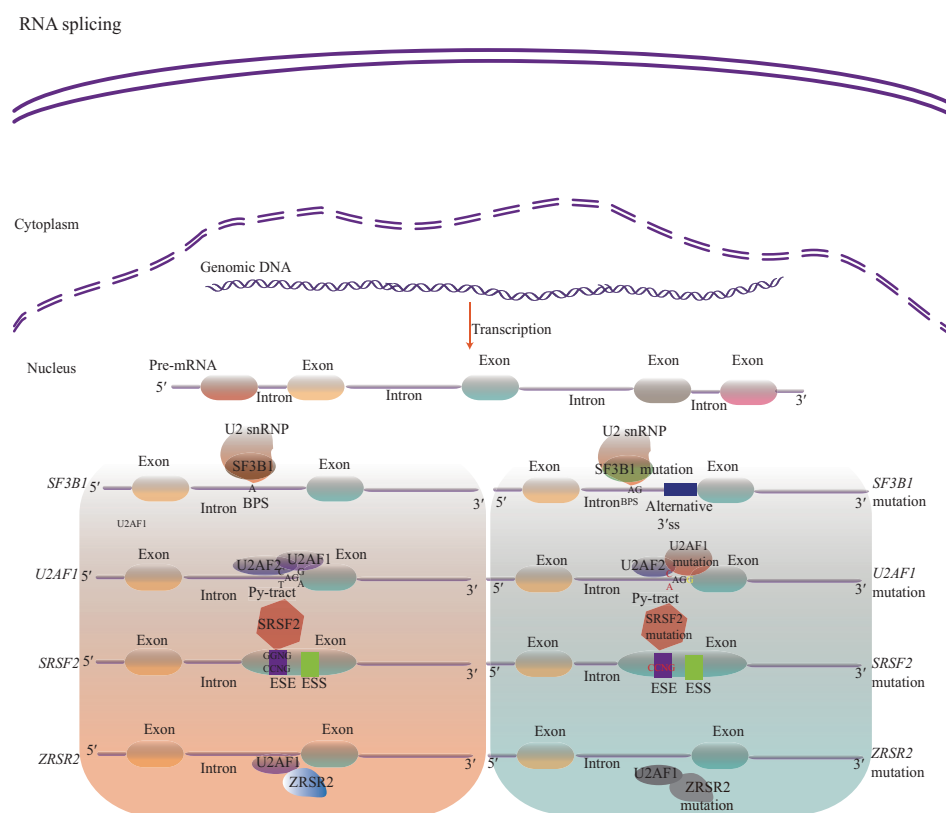
Table 1 The frequency of common gene mutations, prognostic implications, and potential targeted therapies in MDS (modified from the reference [4])

基因 Mutated genes	突变频率 Mutation frequency	预后 Prognostic impact	靶向药物 Targeted therapies ^a
Epigenetic regulators			
<i>TET2</i>	20%	No impact on survival, mutant <i>TET2</i> may predict better response to HMD	HMD
<i>DNMT3A</i>	5%-10%	Unfavourable indicator, especially in <i>SF3B1</i> co-mutated MDS-RARS	HMD
<i>IDH1</i> and <i>IDH2</i>	5%-10%	Unfavourable	Ivosidenib/enasidenib
Chromatin modifiers			
<i>ASXL1</i>	10%-20%	Unfavourable	BAP1 inhibitor
<i>EZH2</i>	10%	Unfavourable	Tazemetostat
Transcription regulators			
<i>RUNX1</i>	15%	Unfavourable	BET inhibitor or Menin inhibitors
<i>ETV6</i>	2%-5%	Unfavourable	N/A
<i>BCOR</i>	5%	Unfavourable	N/A
Cohesion complex components			
<i>STAG2</i>	<10%	Unfavourable	N/A
<i>CTCF</i>	<5%	Unfavourable	N/A
<i>SMC1A</i>	<5%	Unfavourable	N/A
Spliceosome components			
<i>SF3B1</i>	20% in MDS and 65% in MDS-RS	Favourable	Luspatercept or IRAK inhibitors
<i>SRSF2</i>	10%-20%	Unfavourable	N/A
<i>U2AF1</i>	<10%	Unfavourable	IRAK inhibitors
<i>ZRSR2</i>	<10%	Unfavourable	N/A
Signal transduction genes			
<i>JAK2</i>	50% in RARS-T	Unfavourable	Ruxolitinib
<i>KRAS</i>	2%-5%	Unfavourable	Antroquinonol
<i>CBL</i>	2%-5%	Unfavourable	N/A
DNA repair genes			
<i>TP53</i>	5%-10%	Unfavourable	Eprentapopt

HMD: 去甲基化药物; MDS: 骨髓增生异常综合征; MDS-RS: 骨髓增生异常综合征伴环形铁幼粒细胞; MDS-RARS: 骨髓增生异常综合征伴环形铁幼粒细胞难治性贫血; RARS-T: 难治性贫血伴环形铁幼粒细胞增多伴血小板增多; N/A: 无。*表示大多数靶向药物的效果尚未得到证实。HMD: hypomethylating drug; MDS: myelodysplastic syndrome; MDS-RS: MDS with ring sideroblasts; MDS-RARS: MDS with refractory anaemia and ring sideroblasts; RARS-T: refractory anaemia with ring sideroblasts associated with marked thrombocytosis; N/A: not available. ^a notes most targeted agents are of unproven impact.

包括结合未成熟 mRNA(pre-mRNA)上的外显子剪切增强元件(exonic splicing enhancer, ESE), 启动剪切功能; 丝氨酸精氨酸富集结构域的功能主要是与其他蛋白相互作用, 组装剪切复合物。SRSF2同时可以募集转录延长因子 pTEFb^[22-23], 协调转录和剪切的同步高效进行。SRSF2在 MDS 中的突变表现为点突变, 位于 RNA 识别结构域的羧基端, 也是 RRM 和丝氨酸精氨酸富集结构域的链接区, 具体位置位于第 95 位氨基酸, 从脯氨酸(P95)突变为组氨酸(P95H)、氨酸(P95L)或者精氨酸(P95R)^[8,24]。

SRSF2 通常识别 pre-mRNA ESE 中的 SSNG 序列(公共模序序列; 其中 SSNG 中的 S 代表胞嘧啶 C 或鸟嘌呤 G, N 代表任何核苷酸)^[25]。在野生状态下, SRSF2 能无偏好地结合 ESE 中的 CCNG 和 GGNG 公共模序序列^[26]。而突变体 SRSF2 对 ESE 中 SSNG 公共模序序列的识别特异性和连接强度发生变化, 偏向于富含“CC”的位于 RNA 上 ESEs 的公共模序序列(如 CCAG), 从而导致 pre-mRNA 错误剪切^[12,27]。对于突变型 SRSF2, 上述结合力及结合序列特异性(如是否与 5'-CCNG-3' 和 5'-GGNG-3' 有相同的结合偏



RNA剪切复合物SF3B1、U2AF1、SRSF2和ZRSR2突变导致mRNA前体错误剪接(图中右侧图),产生异常蛋白并发挥促癌功能。

Mutations in RNA splicing factors SF3B1, U2AF1, SRSF2, and ZRSR2 resulting in aberrant splicing patterns (right panel) that generate abnormal protein isoforms and drive oncogenic transformation.

图1 RNA剪切复合物的剪接机制(根据参考文献[4]修改)

Fig.1 Splicing mechanism of RNA splicing complex (modified from the reference [4])

好)发生了改变,即突变SRSF2蛋白在与RNA公共模序序列结合时,发生了结合强度和所结合序列特异性的变化,表现为突变型相比野生型SRFS2,与5'-UCCAGU-3'的结合力增加了4.5倍左右;而突变型相比野生型SRFS2,与5'-UGGAGU-3'的结合力不升反而降低^[12]。SRSF2^{P95}突变比SRSF2^{WT}具有更强的NMD诱导活性,以EZH2为例,其错误剪接出一个“有毒”盒式外显子,该外显子引入了一个过早终止密码子,最终导致EZH2的NMD^[12]。WANG等^[28]研究表明,SRSF2^{P95H}突变通过与CSF3R的17号外显子ESE结合,引起异常剪接进而导致CSF3R不同亚型之间的表达比例失衡,促进CSF3R-V4形成,阻滞中性粒细胞分化,从而导致MDS患者的中性粒细胞表型异常。

SRSF2突变细胞需要野生型SRSF2等位基因才能存活,半合子Srsf2^{P95H/-}小鼠相较于杂合子Srsf2^{P95H/+}小鼠,其错误剪接事件发生率高达两倍,且小鼠存在

严重骨髓发育不全,存活时间短^[29]。致死性辐照小鼠接受杂合子Srsf2^{P95H}突变体和纯合子Srsf2缺失的骨髓单核细胞会出现显著的白细胞减少和贫血表型,Srsf2^{P95H}突变的小鼠产生了多系发育障碍,骨髓细胞结构正常,而纯合子Srsf2缺失的小鼠出现骨髓发育不全表型。外周血红系和髓系发育不良也仅见于P95H突变小鼠^[12]。SMEETS等^[30]通过构建Srsf2^{P95H}突变小鼠,证实了Srsf2^{P95H}作为驱动突变出现在造血干细胞可导致突变小鼠出现髓系分化偏移、形态发育不良和单核细胞增多表型,进而使小鼠出现MDS表型。

在对髓系肿瘤进行基因共突变分析时,发现在SRSF2-TET2共突变的患者中,单核细胞比例与共突变的克隆大小呈正相关^[31],XU等^[32]构建了Srsf2/Tet2共突变小鼠,阐释了Srsf2/Tet2共突变较Srsf2单突变或者Tet2单突变会促使明显的骨髓偏倚和单核细胞增多(未成熟的单核细胞、单核细胞和双核单核细胞增多)。

进一步通过对双基因突变细胞进行外显子测序分析,结果显示其突变图谱与人类慢性粒单核细胞白血病中报道的突变图谱相似。将双突变细胞移植于健康受体小鼠,受体出现白细胞增多、单核细胞增多和脾肿大表型。YOSHIMI等^[33]发现在*IDH2*和*SRSF2*双突变细胞中存在剪接异常和整合子复合体成员*INTS3*的表达水平降低,异常的*INTS3*剪接与*IDH2*突变一起促进了白血病的发生,并且依赖于*SRSF2*突变体与*INTS3* mRNA中顺式元件的结合以及*INTS3* DNA甲基化水平的提高。

*Srsf2*纯合子敲除小鼠胸腺细胞损失70%~90%,T细胞分化异常,CD4⁻/CD8⁻ T细胞显著增加,CD4⁺/CD8⁺ T细胞显著减少,此种现象出现的机制涉及*Srsf2*缺失突变促使*Cd45*异常剪接进而影响了胸腺中T细胞的成熟^[34]。*SRSF2*^{P95H}突变的细胞相较于野生型细胞分泌出更高水平的IL-6和IL-8,IL-6影响造血干细胞的发展,导致骨髓细胞的过度产生和MDS患者的异常血液学特征^[35-36]。此外,*SRSF2*^{P95H}突变导致*CASP8*(*CASPASE8*)的6号外显子出现跳跃,从而增强了NF-κB通路的活性^[37]。总之,*SRSF2*^{P95H}突变通过塑造炎症骨髓微环境进而导致MDS发生发展。

*SRSF2*在MDS中的突变频率为12%~15%^[8],*SRSF2*突变与造血髓系偏移、年龄相关的克隆造血、较高的白血病进展率以及较短的临床生存期相关^[38-39]。在低风险MDS患者中,*SRSF2*突变患者的中位总生存时间较*SRSF2*野生型患者显著缩短^[40]。SUTANDYO等^[41]通过Meta分析揭示了*SRSF2*突变与较高的AML转化风险相关。

E7107是一种剪接体抑制剂,通过抑制U2型小核糖核蛋白(small nuclear ribonucleoprotein, snRNP)与mRNA前体连接进而影响剪接体组装^[42]。体外实验显示E7107在*Srsf2*-mut细胞中显著抑制异常剪接,从而减轻小鼠白血病负担^[43]。然而E7107的I期临床试验因受试者出现视神经炎或视力丧失等不良反应而被终止^[44]。

2 *SF3B1*基因突变

*SF3B1*是癌症中最常见的剪接体突变基因,作为U2 snRNP复合物的的重要组成部分参与RNA剪接中3'剪接位点(3' splice site, 3'SS)选择过程中分支点序列(branch-point sequence, BPS)的识别。*SF3B1*

突变主要发生于C末端的HEAT区域(622—781残基),在MDS中的热点突变为K700E、H662Q等^[45]。*SF3B1*突变可能引起mRNA前体的3'端剪接位点异常,从而导致特异性mRNA剪接异常。一方面,*SF3B1*位于U2型snRNP复合物内部,*SF3B1*突变导致U2型snRNP复合物构象发生改变,从而解除对隐性剪接位点的原有空间限制。另一方面,*SF3B1*突变后的U2型snRNP复合物结合的分支位点与野生型的*SF3B1*参与的U2型snRNP复合物结合的分支位点不同,突变的剪接体与分支位点亲和力增高,其结合分支位点后方并可以识别并剪接隐性AG二核苷酸序列,产生异常的mRNA^[46-47]。*SF3B1*突变最终导致细胞中大约50%的异常剪接的mRNA发生NMD,且使相应的基因和蛋白质表达水平下调。*SF3B1*突变也会导致异常的R-loops形成,激活细胞DNA损伤反应增加基因组不稳定性^[48]。

*SF3B1*突变MDS的特征为红细胞减少、中性粒细胞增加、血小板增加、出现RS以及临床疾病进展缓慢^[49],其被归为一类特殊的MDS-SF3B1亚型,诊断标准为MDS骨髓原始细胞<5%,外周原始细胞<2%且伴随有*SF3B1*突变^[5]。*SF3B1*突变MDS导致的红细胞生成异常涉及分化、成熟以及释放等多个方面,如*MAP3K7*被异常剪接进而影响*GATA1*功能,加速红细胞增殖、分化和凋亡^[50];在*SF3B1*基因敲低的红系细胞中,MKRN1大异构体表达水平降低,其异位表达可以挽救红系细胞的生长,并恢复P53及其下游靶点P21、BAX和BBC3的蛋白表达水平^[51]。*SF3B1*突变导致参与血红素合成和线粒体铁运输的基因(*ABCB7*、*TMEM14C*和*ERFE*)剪接异常、红系细胞铁沉积异常,进而导致血红蛋白合成功能障碍和RS形成^[10]。在*SF3B1*突变MDS中也存在铁代谢异常,CLOUGH等^[52]通过构建诱导多能干细胞(induced pluripotent stem cells, iPSCs)模型,首次在体外有效分化了环形铁粒幼细胞,重现了MDS-RS患者的错误剪接模式。在*SF3B1*突变型MDS中,*ABCB7*和*TMEM14C*表达下调协同诱导线粒体铁蛋白显著上调,干扰血红素合成,降低正常骨髓细胞的红细胞生成能力,最终导致RS的形态。*SF3B1*突变骨髓红细胞中存在*ERFE*异常剪接所产生的突变蛋白,其与常规的*ERFE*蛋白均可抑制铁调素的功能,最终导致铁过载。

*SF3B1*在免疫系统中起着至关重要的作用,

通过各种复杂的机制塑造MDS中的炎症微环境。CHOUDHARY等^[53]发现*SF3B1*^{K700E}突变导致MDS中*IRAK4*外显子6异常保留,产生*IRAK4-L*亚型,过度激活下游NF- κ B信号,塑造了MDS的炎症微环境。POLLYEA等^[54]证明*SF3B1*突变增强了巨噬细胞、患者来源的细胞系、小鼠和人骨髓细胞中NF- κ B的活性并促进了LPS诱导的炎症细胞因子的产生。*SF3B1*突变患者来源的单核细胞产生的S100A8、IL-6较野生型更多,IL-6在炎症相关癌症中发挥至关重要的作用。因此,*SF3B1*突变导致的免疫异常可能会改变免疫细胞功能,从而使MDS患者感染风险增加或者出现过度炎症反应。

HUBER等^[55]对734例MDS患者进行分析,其中31%患者存在*SF3B1*突变,*SF3B1*突变型相较于野生型MDS有相对较长的总生存时间和较低的AML转化率。DALTON等^[56]和KANAGAL-SHAMMANNA等^[57]对*SF3B1*的突变亚型进行预后分析发现*SF3B1*突变患者预后良好,其中*SF3B1*^{K700E}突变MDS患者预后良好,*SF3B1*^{K666N}突变与高危、血小板减少和较差的预后有关。SONG等^[58]对95例MDS进行分析发现,与仅携带*DNMT3A*单突变的患者相比,*SF3B1*/*DNMT3A*共突变的患者呈现出更有利的细胞遗传学特征,同时*SF3B1*/*DNMT3A*突变患者的中位生存时间和总生存期明显长于单纯*DNMT3A*突变患者。

罗特西普在治疗低风险MDS患者贫血方面具有良好的耐受性和有效性,并被批准用于治疗伴有RS和/或*SF3B1*突变的输血依赖性低风险MDS患者^[59-60]。此外,最近的研究发现罗特西普具有刺激成骨细胞成熟的能力,并可能改善伴有贫血和骨质流失的MDS^[61]。*SF3B1*突变的MDS患者对红细胞生成素的反应较野生型患者更加敏感,*SF3B1*突变伴随5q-的MDS患者在免疫表型和细胞谱系方面更接近于5q-的MDS患者,因此*SF3B1*突变伴随5q-的MDS患者在选择来那度胺疗效较好^[62]。*SF3B1*作为治疗靶点的研究一直在进行中,其中Jerantinine A是一种新型吲哚类生物碱,通过抑制微管蛋白聚合,上调*SF3B1*和*SF3B3*表达水平,诱导G₂/M细胞周期阻滞和肿瘤特异性细胞死亡,具有有效的抗肿瘤细胞增殖活性^[63]。H3B-8800作为一种非常有前景的口服SF3B复合物调节剂,可以优先杀死剪接体突变的血液和上皮肿瘤

细胞,在晚期髓系恶性肿瘤患者的I期临床试验中显示出令人满意的安全性和临床疗效^[64]。OTS964是一种高选择性CDK11抑制剂,可以阻断*SF3B1*剪接体激活的关键步骤^[65]。*SF3B1*突变MDS患者存在DNA损伤反应的异常激活,这种DNA损伤反应使携带*SF3B1*^{K700E}突变的肿瘤细胞对小分子抑制剂(如PARP抑制剂)敏感^[66]。因此,针对剪接体突变下游事件的药物开发为治疗*SF3B1*突变型MDS提供了可行的策略。

3 U2AF1突变

U2AF1与U2AF2共同形成U2AF复合物,此复合物识别U2内含子的3'SS并且招募U2 snRNPs^[67]。U2AF1结构域包括一个U2AF同源基序、一个富含丝氨酸/精氨酸基序以及两个保守的CCCH型锌指(ZnF1和ZnF2),同源基序可以与U2AF2形成二聚体,锌指结构可以结合靶RNA的3'SS内含子-外显子边界^[68-69]。杂合热点突变分别影响ZnF1和ZnF2结构域内的S34和Q157残基,导致造血关键基因的序列依赖性错误剪接。先前有报道称,S34突变优先导致3'SS区-3位置上带U的外显子被排除,而在同一位置上包含带C的外显子。Q157突变优先排除以A开头的外显子,包含3'SS区+1位置以G开头的外显子^[17,70-71]。也有研究认为U2AF1突变体对排除外显子或保留内含子的剪接连接处亲和力减低^[72]。然而,导致这些异常剪接并最终导致疾病的分子机制仍需要进一步研究。

在MDS中*U2AF1*的突变频率为5%~21.7%^[73],野生型的*U2AF1*参与维持造血干/祖细胞的生存以及正常功能,体外研究表明*U2AF1*^{S34F}突变的造血干/祖细胞存在凋亡增加、生长抑制以及红系分化障碍,*U2AF1*^{S34F}突变也可损害粒单核细胞正常生长和分化功能,使其更多分化为粒细胞(以嗜酸性粒细胞为主)^[74]。SHIRAI等^[70]构建的*U2af1*^{S34F}转基因小鼠出现了骨髓和脾脏中造血祖细胞增加,骨髓中成熟造血谱系分布改变,单核细胞和B细胞减少和中性粒细胞增加表型。DUTTA等^[75]构建的*U2af1*^{S34F}转基因小鼠出现全血细胞减少、骨髓衰竭表型。综上所述,已有的细胞模型和小鼠模型证明了*U2AF1*^{S34F}突变可能导致造血干细胞的严重缺陷,但是上述2种小鼠模型均未出现人类MDS的全部表型,提示*U2AF1*基因突变可能不足以驱动人类MDS的发生。在机制方面,YIP等^[74]和KIM等^[76]发现*U2AF1*^{S34F}突变导致*H2AFY*

和STRAP基因异常剪接进而损害了红系、粒系和淋系的正常分化。PARK等^[20]发现U2AF1^{S34F}突变可降低ATG7蛋白水平导致自噬缺陷、线粒体功能障碍和继发致癌突变频率增加。对1 700例MDS患者的分子突变和临床数据进行分析,结果表明与U2AF1共突变的基因主要是转录因子相关基因和DNA甲基化基因,包括ASXL1、BCOR、TET2、DNMT3A、PHF6、ETV6、RUNX1、STAG2和SETBP1。在U2AF1突变MDS患者中,ASXL1和RUNX1突变可能增加白血病转化和疾病复发的风险^[77-78]。

MDS细胞中的U2AF1^{S34F}突变可促进NLRP3炎症小体依赖性IL-1 β 和IL-18的产生,塑造骨髓炎症微环境,引发造血干细胞焦亡^[79]。U2AF1突变可直接介导产生具有致癌活性的较长的IRAK4-L蛋白亚型,介导NF- κ B和MAPK通路激活,维持白血病细胞的功能。在IRAK4-L高表达或U2AF1突变的AML细胞中抑制IRAK4-L的表达可显著抑制白血病细胞生长^[80]。

U2AF1突变对MDS预后的影响尚存争议,LI等^[19]对511例MDS患者中的U2AF1突变特征进行分析发现,相较于野生型U2AF1患者,突变型U2AF1患者血红蛋白和血小板水平显著降低,拥有更多的8号染色体三体异常和较少的复杂核型。U2AF1突变与MDS患者较短的总生存时间相关,进一步进行突变亚型分析,结果提示在高危患者中携带U2AF1^{S34}突变的患者生存率较低。GRAUBERT等^[17]对150例MDS患者的突变与预后情况进行分析,发现U2AF1突变与AML快速进展相关,但与野生型U2AF1患者相比较,U2AF1突变对总生存时间和无病生存时间无显著影响。WANG等^[81]对3 322例MDS患者进行Meta分析,结果显示U2AF1突变与较短的总生存期以及较多的AML转化有关。同时也有研究认为U2AF1突变与MDS预后无关^[82]。这些研究结果之间的差异可能与病例样本数、种族遗传背景、随访时间等有关。

舒德霉素(Sudemycin)是一种调节mRNA前体剪接的化合物,其对U2AF1突变的髓系肿瘤患者有潜在的作用,在体外研究中发现U2AF1^{S34F}突变的细胞对舒德霉素敏感,体内研究表明通过舒德霉素处理U2af1^{S34F}突变小鼠可以抑制突变的造血干细胞扩增^[83]。调节U2AF1^{S34F}下游靶基因H2AFY和STRAP的异构体表达可以挽救由U2AF1突变引起的MDS细胞红系分化缺陷,说明剪接调节剂可能对U2AF1突变

的髓系肿瘤有效^[76]。在U2AF1^{S34F}突变的AML细胞中敲低靶基因IRAK4-L或CA-4948可以增强红细胞和髓细胞的分化并抑制THP1细胞异种移植小鼠的白血病细胞生长^[80]。

4 ZRSR2突变

ZRSR2(也被称为URP)位于X染色体(Xp22.1)上,编码参与识别3'内含子剪接位点的剪接因子,ZRSR2与U2AF2/U2AF1异源二聚体和SRSF2剪接组分组成剪接体。体外剪接实验表明,ZRSR2是有效剪接主要和次要内含子的必要条件^[84]。野生型的ZRSR2以ATP依赖的方式被募集到U12型内含子剪接位点,促进剪接体复合物形成;或者ZRSR2于mRNA剪接的第二步被连接于U2型内含子的3'剪接位点^[84]。在MDS中,ZRSR2无热点突变,突变可以发生在ZRSR2转录本的任何位点,这与在SF3B1、SRSF2和U2AF1的热点突变位点不同。此外,ZRSR2基因在男性中常发生无义突变、剪接位点突变和移码突变,表明其为功能丧失型突变,ZRSR2表达水平降低会抑制细胞生长并改变造血细胞的体外分化潜能,使髓系分化增加和红系分化减少^[85]。MADAN等^[86]构建的Zrsr2缺失小鼠的造血干细胞保留多系重建能力,提示Zrsr2在小鼠造血中的功能有限,但是此结果可能是Zrsr1表达对Zrsr2缺失进行了代偿导致,而ZRSR1在人体中并无表达。因此单纯的Zrsr2缺失小鼠并不能重现人ZRSR2突变MDS表型。在机制方面,ZRSR2突变会导致MAPK9和MAPK14的内含子保留导致含有过早终止密码子的异常转录本的产生,MAPK9和MAPK14蛋白水平降低,进而影响它们在造血发育中的功能^[86]。MADAN等^[86]验证影响造血细胞分化或MDS进展的重要基因,发现WDR41、FRA10AC1和SRPK2基因中的U12型内含子均出现了错误剪接。ZRSR2突变导致IRF7内含子保留,IRF7表达量降低,进而使浆细胞样树突状细胞受到炎症刺激后的细胞激活和细胞凋亡信号通路失调,最终损害机体免疫力并促使白血病转化^[87]。ZRSR2经常与表观遗传调节基因TET2出现共突变,GARCIA-RUIZ等^[88]构建了Zrsr2^{m/m}Tet2^{-/-}突变小鼠,小鼠出现了外周血细胞减少、脾肿大、髓外造血和造血干祖细胞发育异常的MDS表型,同时其中骨髓中炎症因子IFN- γ 、IL-6、IL-10和TNF- α 表达水平升高,骨髓微环境呈现高炎症状态。

*ZRSR2*突变在无环形铁粒幼细胞的MDS亚型和慢性粒单核细胞白血病中发生频率更高,并且与中性粒细胞减少、骨髓原始细胞百分比升高和AML进展率升高相关^[89]。研究人员对221例MDS预后进行分析,发现*ZRSR2*突变患者主要分布在IPSS评分中的中危-1和中危-2组,*ZRSR2*突变且*TET2*野生型患者的预后差且AML转化率高^[10,85,89]。但是THOL等^[82]对193例MDS患者进行分析,发现*ZRSR2*野生和突变患者总生存时间和AML转化率之间无显著差异。因此,需要在临床数据中进一步评估*ZRSR2*突变对MDS预后的影响。

5 总结与展望

由于体内超过90%的未成熟mRNA都要通过RNA剪切复合物的剪接才能成为正常成熟的mRNA,进而被运送到胞质进行转录和翻译,所以RNA剪接因子作为驱动突变或重要突变会影响多种下游基因的表达、改变多条信号通路的活性或重塑骨髓造血微环境进而影响造血干祖细胞的功能和分化,因此其在MDS的发生发展、治疗决策以及预后评估中扮演了重要角色。

*SRSF2*突变与MDS患者的较短的生存期以及较高的白血病进展率密切相关,*SRSF2*突变不仅会直接干扰正常的RNA剪接过程导致NMD,同时也会影响MDS患者的骨髓微环境,导致NF- κ B信号通路活性增强,IL-6、IL-8等炎症因子过度分泌,塑造出骨髓炎性微环境,但是目前尚待开发靶向*SRSF2*突变的药物。*SF3B1*的不同突变状态与MDS的不同预后相关,罗特西普已被批准用于*SF3B1*突变的输血依赖性低风险MDS患者,其临床获益是否与*SF3B1*突变类型有关尚需要进一步临床试验或临床数据分析进行确认。靶向*SF3B1*的药物H3B-8800已进入临床试验阶段并取得初步的结果,但其作用机制以及不良反应等需要进一步研究揭示。*U2AF1*突变与MDS的预后尚存争议,临床前研究提示舒德霉素靶向*U2AF1*^{S34F}突变抑制*U2AF1*突变导致的造血干祖细胞扩增,但是其有效性和安全性还缺乏进一步的临床研究。*ZRSR2*突变对MDS预后的影响尚且存在争议,目前暂无靶向*ZRSR2*的临床前以及临床研究。多项研究表明,*SRSF2*、*SF3B1*、*U2AF1*以及*ZRSR2*突变可通过激活NF- κ B等信号通路增加炎症因子如IL-6、IL-8、IFN- γ 、IL-1 β 等的表达水平,从而塑造

MDS炎症微环境,但目前炎症因子拮抗剂如托珠单抗、卡那单抗等调节免疫微环境是否可以改善MDS患者的预后尚且需要进一步开展高质量的临床试验进行明确。剪接因子调节下游多条通路如ATR-CHK1、MAPK以及NLRP3等,因此联合使用下游通路调节剂可能是一种潜在的有效的治疗MDS的手段。

随着分子机制研究手段的进步以及人工智能的发展,进一步对剪接因子突变的致病机制进行明确以及利用人工智能开发靶向剪接突变的新药或筛选有效的老药迫在眉睫,同时进一步改造因不良反应终止临床试验的靶向药物使其更加高效低毒从而应用于临床也是目前需要解决的问题之一。总之,针对剪接因子突变的致病机制进而开发MDS的治疗药物,以及制定高效低毒的治疗策略来管理高风险、复发和初治耐药的MDS患者是目前需要共同努力的方向。

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