急性 B 淋巴细胞白血病发病机制研究进展

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摘要 急性B淋巴细胞白血病(B-ALL)是由于B淋巴细胞发育和增殖相关基因等突变, 阻碍了B淋巴祖细胞的正常分化和成熟, 伴随着异常增殖而引起的克隆异质性疾病, 是高发于儿童的一种主要血液系统恶性肿瘤. 基于对已有基因突变导致的B-ALL发病机制的深入理解和研究, B-ALL的治愈率在儿童患者中达80%以上, 但仍有约20%的儿童患者和60%的成人患者最终复发. 近年来, 利用基因芯片和二代测序等技术在复发的B-ALL患者检测到很多新的基因突变类型, 但是这些基因突变与其他协同融合基因在B-ALL中的具体发病机制仍不明确. 本文主要从涉及细胞发育分化、表观遗传调控、细胞周期和凋亡、细胞信号转导通路、非编码RNA等协同基因突变以及白血病干细胞方面分析B-ALL发生和耐药复发的机制, 并总结分析目前常用的B-ALL相关小鼠(*Mus musculus*)模型, 以期对复发性B-ALL的发病机制、风险分级和治疗策略等给予全新的认识.

关键词 B-ALL, 融合基因, 协同基因, 复发, 白血病干细胞, 小鼠模型

急性淋巴细胞白血病(acute lymphoblastic leukemia, ALL)是儿童常见的恶性肿瘤, 且发病年龄集中在2~5岁, 多是由于相关基因突变导致淋巴干祖细胞恶性增殖并伴有不同程度的发育分化受阻而导致的遗传异质性疾病. 根据肿瘤细胞的免疫表型可以将ALL分为急性B淋巴细胞白血病(B-cell precursor acute lymphoblastic leukemia, B-ALL)和急性T淋巴细胞白血病(T-cell precursor acute lymphoblastic leukemia, T-ALL). 大约75%的B-ALL患者经常可以发现染色体数目异常或者染色体易位^[1], 其中染色体数目异常包含超二倍体和亚二倍体(表1). 尽管高二倍体发生的生物学机制并不明确, 但是其预后相对于低二倍体较好. 染色体易

位可以导致融合基因的发生, 主要包含*ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, *MLL*相关融合基因^[2,3]. 早期融合基因突变的产生进而干扰了造血相关基因、癌基因、抑癌基因或者酪氨酸激酶等基因的正常表达, 促使B-ALL发生. 通过对融合基因致病的深入研究, B-ALL的治愈率在儿童中可达80%左右, 但是仍有20%的儿童患者和60%的成人患者治愈率相比较差, 存在复发风险. 通过对这些复发难治的B-ALL患者进行分析发现, 多种协同基因的突变模式可以加速B-ALL发生进程, 同时也参与疾病复发. 在过去的10年间人们利用高通量基因芯片和二代测序等先进手段对含有相关融合基因突变的B-ALL患者分析后, 一批新的基因

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表1 B-ALL患者中常见的染色体异常所引起的致癌基因分类

染色体异常	融合基因	临床预后	发病率	PMID号
Hyperdiploidy (大于50条染色体)	—	预后较好	儿童占30%; 成人占9%	27308574
Hypodiploidy (小于44条染色体)	—	预后差	5%~8%	15147369
t(12;21)(p13;q22)	<i>ETV6-RUNX1 (TEL-AML1)</i>	预后差	儿童占25%; 成人占1%~4%	7492786; 9332479
t(1;19)(q23;p13)	<i>TCF3-PBX1 (E2A-PBX1)</i>	预后差	儿童占6%; 成人占3%	2380759; 7989935; 9469337; 20010622
t(17;19)(q22;p13)	<i>E2A-HLF</i>	预后差	<1%	1386162; 21606506
t(9;22)(q34;q11.2)	<i>BCR-ABL1</i>	预后差	儿童占5%; 成人占30%	3307953; 8839828; 3541203; 3332859
t(4;11)(q21;q23)	<i>MLL-AF4</i>	预后差	儿童占2%; 成人占9%	11986937; 8481519
t(9;11)(p21;q23)	<i>MLL-AF9</i>			9359508; 17170120
t(10;11)(p12;q23)	<i>MLL-AF10</i>			19825449; 20592248
t(11;19)(q23;p13.3)	<i>MLL-ENL</i>			9054669; 8605327

突变类型被逐渐发现. 这些突变基因在B-ALL的发生和复发过程中扮演了重要的角色, 主要涉及B细胞发育调控、细胞周期和凋亡、细胞增殖和存活、细胞自噬、表观遗传调控等相关基因(表2)^[4-9]. 这些后期突变的协同基因具有重要的致病作用, 其中一些突变模式在特定的融合基因中存在, 也可能与复发耐药克隆或者白血病干细胞(leukemia stem cells, LSC)相关. 本文主要通过对这些新的基因突变模式和目前所应用的相关B-ALL小鼠(*Mus musculus*)模型等分析归纳, 以期对B-ALL的发生和复发机制有新的认识, 最终为B-ALL的精准诊治提供新的干预策略和治疗靶点.

1 临床B-ALL患者中常见的基因突变

临床B-ALL患者中发现的基因突变主要包含基因融合突变和基因协同突变, 前者主要为早期突变事件, 单独存在不足以诱发完整的B-ALL的产生, 且发现融合基因突变的时间也各有不同, 如*MLL*融合突变较*ETV6-RUNX1*, *BCR-ABL1*等突变要出现得早一些, 出现较晚的融合基因需要的协同基因突变数目较多^[10]; 后者主要为补充协同事件, 可以提高患者的发病和复发风险, 或者加速B-ALL的发病进程, 甚至有时在无融合基因突变的情况下也可以单独诱发B-ALL. 不同的融合基因突变和协同基因突变类型可以分别参与B

细胞发育、细胞增殖、凋亡、自噬、表观遗传调控等重要事件, 进而促使诱发不同类型的B-ALL. 这些新的突变类型或许可以为目前治疗效果不佳的患者提供新的治疗靶点和预后分析.

1.1 常见的基因突变模式

(1) 融合基因突变. *ETV6-RUNX1 (TEL-AML1)*融合基因的产生是由于染色体易位t(12;21)(p13;q22)导致的, 通过荧光原位杂交(fluorescent in situ hybridization, FISH)技术发现25%左右的B-ALL儿童患者含有此类突变, 但在成人中相当少见(表1)^[11,12]. 此类突变通过FISH和RT-PCR很容易被检测到^[3,13], 而且治愈率较其他类型的B-ALL要高很多^[14]. 但是至今, *ETV6-RUNX1*, *BCR-ABL1*, 超二倍体等突变在中国地域的儿童患者中仍然是一个预后不佳的重要因素^[15]. 众所周知, *ETV6*和*RUNX1*是造血发育过程中所必需的转录调控因子, 这类基因的异常表达在多种类型的血液肿瘤中经常被检测到^[16,17], 在iAMP21 (intrachromosomal amplification of chromosome 21)患者中就发现*RUNX1*的拷贝数大于3, 且患者预后极差^[18]. 那么二者的融合突变是怎么导致B-ALL的发生呢? 研究发现, *ETV6-RUNX1*可直接抑制*RUNX1*下游靶基因的表达, 驱动B-ALL的发生^[19]. *ETV6-RUNX1*还可以直接结合在*EPOR*的启动子上, 促进*EPOR*的mRNA表达水平, 而

表2 协同基因突变在B-ALL发生过程中的调控作用分类

细胞功能	相关协同基因	基因突变类型	PMID号
B细胞发育	<i>PAX5, EBF1</i>	功能缺失突变	26301816; 23684985
	<i>BCL6, BACH2, GATA3, Zfp521</i>	功能获得突变	25780007; 25759025; 22858985; 23852341; 21593590; 23684985; 28415601
细胞周期	<i>CDKN2A/B, p53</i>	功能缺失突变	26301816; 24503810
	<i>BMI1</i>	功能获得突变	24503810
细胞凋亡	<i>Aurora kinase A and B, Survivin</i>	功能获得突变	22940834
	<i>CASP7</i>	功能缺失突变	24503810
细胞自噬	<i>Beclin1, Atg7, Atg5, LC3, p62</i>	功能缺失突变	25615280
细胞信号通路	<i>JAK1, JAK3, CRLF2, IL7R; NRAS, KRAS, PTPN11; pre-BCR, AKT phosphorylation, Wnt1, Wnt16</i>	功能获得突变	26301816; 23153538
	<i>ROR1, SERCA3</i>	功能缺失突变	23153538; 28651627
细胞表观遗传调控	<i>p73, MGMT</i>	DNA 甲基化	12970785
	<i>GCN5, PCAF</i>	蛋白乙酰化	23044487
	<i>miR-126, miR-222, miR-339, miR-142-3p</i>	microRNA上调	19206004; 21701489; 20549547; 19435910
	<i>hsa-miR-451, hsa-miR-373, miR-196b, miR-124a, miR-143, miR-509</i>	microRNA下调	21706045; 27300437; 21242186; 24153013

EPOR被激活后, 进而通过激活JAK2-STAT5信号通路和上调抗凋亡因子BCL-XL等参与细胞的生存状态调控^[20]。然而ETV6-RUNX1的驱动诱发B-ALL的病理机制似乎不是非常明确, 或者此类患者应用传统的治疗方案效果不佳, 似乎存在未知的突变类型参与上述事件发生。最近通过全外显子测序携带ETV6-RUNX1突变的细胞发现, 白血病发生相关的基因SP140, GATA2, SMAD5, KMT2C, CDH17, CDX2, FLT3, PML, MOV10L1等均存在相应的突变^[9]。由此可见, ETV6-RUNX1突变可以通过调控细胞发育、细胞增殖和凋亡事件参与诱发B-ALL。

BCR-ABL1融合突变是由于染色体易位t(9;22)(q34;q11.2)导致费城染色体(Philadelphia chromosome, Ph)的形成而产生, 此类突变在成人和儿童中分别占25%~30%和3%~5%(表1)^[21]。由于BCR存在两种断点区, 因此BCR-ABL1融合基因可以编码两种融合蛋白: 210和190 kD。前者的BCR断点区出现在外显子13和14或者14和15之间, 参与慢性粒细胞白血病(chronic myelogenous leukemia, CML)的发生; 而后者BCR的断点区出现在外显子1和2之间, 在90%的BCR-ABL1阳性B-ALL中被发现^[22-24]。BCR-ABL1融合突变导致酪氨酸

激酶持续激活, 进而激活多种信号通路, 促进细胞增殖, 抑制细胞分化和黏附等^[25]。在BCR-ABL细胞中发现, IK6 (dominant-negative Ikaros isoform 6)可以激活STAT5和HO-1 (heme oxygenase-1), 促进B-ALL细胞存活。BCR-ABL由于含有持续的酪氨酸激酶活性, 此类患者对酪氨酸激酶抑制剂(tyrosine kinase inhibitors, TKIs) imatinib mesylate敏感, 但是在含有IKZF1缺失突变时, BCR-ABL1突变导致的B-ALL对TKIs产生耐药性并存在复发风险^[26-30]。B-ALL临床患者中还发现一类基因表达谱与BCR-ABL1突变患者十分相似, 但是并无BCR-ABL1突变存在, 但是在这类BCR-ABL1-like患者中检测到IKZF1突变, 通过转录组测序和外显子测序分析, 发现细胞因子受体和激酶信号通路信号被大量激活, 涉及的成员主要包含ABL1, EPOR, IL7R, JAK2, PDGFRB等^[28]。但是目前所应用的酪氨酸激酶抑制剂在部分患者中出现耐药性, 提示需要根据患者的自身情况设计更加敏感的药物或者发掘其他协同突变基因靶点。

TCF3-PBX1 (E2A-PBX1)融合突变是由于染色体易位t(1;19)(q23;p13)而产生的, 在儿童和成人中分别占6%和1%~3%(表1)^[31-34]。TCF3编码E12和E47两个转

录因子, *PBX1*编码一个同源结构域蛋白成员,二者参与淋巴细胞发育调控过程. *TCF3-PBX1*包含*TCF3*的转录激活结构域和*PBX1*的DNA结合结构域. *TCF3-PBX1*可以通过干扰*HOX*和*TCF3*靶基因的表达,进而干预造血调控过程^[35,36]. 研究发现, *TCF3*还可以通过染色体易位(17;19)(q22;p13),和锌指转录因子*PAR*家族成员*HLF*融合形成*TCF3-HLF*融合蛋白, *TCF3-HLF*可以调控淋巴祖细胞中细胞死亡相关基因*LMO2* (LIM domain only 2)和*BCL2* (B-cell CLL/lymphoma 2)的表达水平^[37]. 数据显示, preBCR⁺的E2A-*PBX1*细胞中, E2A-*PBX1*能直接结合在*ZAP70*, *SYK*和*LCK*的启动子处, 激活其表达, 从而使PLC γ 2过度磷酸化被激活, 促使细胞恶性增殖^[38]. 近年发现, *Meis1*可以与*PBX1*, *HOXA9*形成共转录因子, 介导*Hif-1 α* 的转录激活, 参与HSC的能量代谢过程^[39]. 另外发现异常情况下的E2A-*PBX1*的TALE同源域可以和*HOXA9*协同结合, 直接靶向激活*Flt3*, 同时抑制B细胞发育分化^[40,41], 提示E2A-*PBX1*通过能量代谢途径参与B-ALL的发病进程. 由此可见, E2A-*PBX1*可以通过调节B细胞发育, 代谢和凋亡等事件诱发B-ALL, 针对此类靶向药物的开发或许能使此类患者受益.

MLL (也称KMT2A, the lysine (K)-specific methyltransferase 2A)编码的蛋白参与组蛋白甲基转移酶活性, 而组蛋白甲基转移酶可以通过调节正常同源基因的表达参与造血调控过程^[42]. 因此, *MLL*相关融合突变可以通过异常的表现遗传调控方式参与B-ALL的发生. *MLL*相关融合突变占婴儿B-ALL的2/3, 儿童B-ALL的1%~2%, 成人B-ALL的4%~9%, 且预后极差(表1)^[43~50]. *MLL*相关融合突变含有*MLL*的DNA结合结构域和甲基转移酶活性. t(4;11)(q21;q23), t(9;11)(p21;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.3)等染色体易位可以导致*MLL*分别与*AF4*, *AF9*, *AF10*, *ENL*融合^[51,52]. 此类融合蛋白可以和DOT1样组蛋白H3K79甲基转移酶DOT1L互作, 改变*MLL*靶基因*HOXA9*的组蛋白甲基化模式, 进而异常的*HOX*表达水平导致HSC的自我更新和增殖能力受到干扰^[53~55]. 由于B细胞异常发育时重组酶的活性异常升高, 导致*MLL*的融合突变不断增加, 例如, 近来通过二代测序发现*MLL*可以和*MAML2*融合导致B-ALL发生^[56]. *MLL*融合突变和其他融合基因一样, 也不同程度的需要额外的协同基因突变, 例如, 近来发现的*PI3K*, *KRAS*, *NRAS*, *FLT3*, *NF1*,

*PTPN11*等激活突变可以使*MLL*突变的细胞更容易恶性扩增^[57].

面对传统的治疗策略无效的情况下, 罕见融合基因在疾病早期突变阻碍了B细胞分化发育以及参与细胞自我更新, 表观遗传调控等事件, 进而驱动的B-ALL的发生近年来逐渐受到人们的高度重视. 例如, 在发病早期, *DUX4*可以易位到*IGH*位点导致*DUX4-IGH*的产生, 使得*DUX4*过表达, 而且这种融合模式常常伴随着*ERG*的缺失突变, 其表达的突变体*ERGalt*能抑制野生型*ERG*的活性, 进而参与白血病的发生. 研究发现, 当*ERG*突变患者预后较好, 但是当和*IKZF1*突变同时存在时, 患者预后往往不佳^[58]. 与*DUX4*融合突变一起早期出现的还有*ZNF384*和*MEF2D*等的融合突变模式, 例如, 临床已经发现的*EP300-ZNF384*, *TAF15-ZNF384*, *SYNRG-ZNF384*, *MEF2D-DAZAP1*, *MEF2D-BCL9*, *MEF2D-HNRNPUL1*等融合基因的表达, 可以使得B细胞分化受阻, 组蛋白去乙酰化酶HDAC9升高, 使得部分小鼠发生B-ALL, 可能这些早期突变事件还不完全足以促进B-ALL的充分发生, 需要累积更多的其他基因突变类型. 且发现*DUX4*, *ZNF384*和*MEF2D*这三类融合基因突变多集中在Ph⁺类型的成人B-ALL患者中, 也从侧面验证了这种融合基因需要累积更多的基因突变才能导致B-ALL, 未来针对这种特点的融合突变模式的发病规律, 或许可以使得这部分患者得到针对性的风险分级和治疗方案^[59~61].

染色体数目增多或者减少可以导致相关癌基因和抑癌基因的表达失常, 进而引发B-ALL. Hyperdiploidy是由于常规染色体中至少5条染色体随机获取导致的染色体数目大于50, 大约占儿童患者的25%~30%, 成人患者的9%(表1)^[62]. 有超过1/2的B-ALL患者染色体异常增多主要集中在染色体4, 6, 10, 14, 17, 18, 21, X等^[63]. 此突变在儿童的治愈率相比成人要好, 可能是由于成人累积了其他基因突变导致B-ALL耐药或者复发风险较高^[49,50]. 如累积了*NRAS*, *KRAS*, *CREBBP*突变的Hyperdiploidy预后较差^[64,65]. 研究还发现, 复发相关的染色体异常主要涉及5和17^[66,67]. Hypodiploidy是常染色体缺失导致其数目少于44条, 大约占B-ALL患者的5%~8%左右, 一般预后较差^[68]. 根据患者的染色体数目缺失程度可以分为4种类型: 近单倍体(24~31条)、低亚二倍体(32~39条)、高亚二倍体(40~43条)、近二倍体(44或者45条). 其中低二倍体患者经常含有*IKZF3*

和*p53*突变, 可以作为预后较差的指标^[69].

(2) 协同基因突变. 尽管以上融合基因突变模式在B-ALL的起始发病过程中非常重要, 但是其单独存在并不足以诱发B-ALL表型^[70], 甚至在不*BCR-ABL*融合基因存在下, 单凭*IKZF1*, *CRLF2*, *PDGFRB*, *ABL1*, *JAK2*, *EPOR*, *SH2B3*, *IL7R*等突变也可导致*BCR-ABL*-like表型的B-ALL发生^[28,71,72], 说明除了早期的融合基因突变驱动B-ALL发生外, 其他的协同突变模式是B-ALL逐步发生和复发的关键, 这也符合肿瘤发生的多基因突变理念. 此类假说在*ETV6-RUNX1*患者中得到证实, 例如, *ETV6-RUNX1*在B祖细胞的表达并不足以诱发B-ALL发生, 且在健康儿童中也有检测到*ETV6-RUNX1*突变^[70,73,74]. 高通量基因芯片数据也证实, *ETV6-RUNX1*患者含有的B细胞发育相关的转录调控因子*PAX5*和*EBF1*存在功能缺失突变. 且此类协同基因的突变也使得B-ALL的发病风险明显上升, 例如, 儿童患者中发现的*IKZF1*, *ARID5B*, *CEBPE*, *CDKN2A*等突变是目前认为的高发易感致病因素^[75-77]. 因此, 通过高通量芯片分析和二代测序手段(靶向测序、外显子测序、转录组测序、全基因组测序), 人们陆续发现在B细胞发育、细胞周期与凋亡、细胞自噬和表观遗传调控等相关基因突变在B-ALL二次打击模式中扮演着重要的角色(表2)^[4,6].

1.2 常见突变基因参与的细胞功能

(1) B细胞发育相关基因突变. 造血干细胞(hematopoietic stem cells, HSCs)分化为B细胞的过程是一个HSC多向分化和自我更新能力丢失, 并且伴随B细胞特异基因逐渐表达的精密复杂事件, 除了涉及细胞自身的很多转录调控因子如*RUNX1*, *IKZF1*, *E2A*, *EBF1*, *PAX5*等调节外, 还受到骨髓微环境中*IL-7*, *SCF*和*FLT3*的精准调控, 进而驱动*V(D)J*基因重排影响B细胞表达BCR (pre-B cell receptor)^[78-80], 还有一些如*STAT5*等抑制凋亡基因*MCL1*的调控蛋白维持B细胞的存活状态^[81], 从而避免机体B细胞发育过程异常(图1). 目前发现有近2/3的B-ALL患者含有B细胞发育相关的基因点突变, 片段缺失或者基因易位导致的基因功能缺失, 如*PAX5*, *EBF1*, *IKZF1*, *LMO2*等功能缺失^[82-85]. 其中以*PAX5*, *IKZF1*突变频率最高^[4], 且发现*PAX5*和*IKZF1*的结构异常突变多于基因序列缺失突变. 由于B细胞的发育分化受阻且恶性增殖, 因此大多

数B-ALL免疫表型为B祖细胞的标志分子.

*PAX5*是机体正常造血发育分化的关键转录调控因子, 它促进B细胞成熟发育的同时会抑制T细胞和髓系细胞的特异蛋白表达, 因此*PAX5*缺失突变可以导致大量B祖细胞异常累积. *PAX5*的点突变, 片段缺失和基因易位在B-ALL患者中较为常见, 导致正常的*PAX5*失去完整的DNA结合结构域或者保留相对完整的转录激活结构域, 引起*PAX5*单倍体剂量不足(haplo-insufficiency)^[86]. 目前为止, *PAX5*相关的融合伙伴基因主要包含*ETV6*, *ELN*, *POM121*, *PML*, *FOXP1*, *MLLT3*, *JAK2*, *C20orf112*, *AUTS2*, *CHFR*, *SOX5*, *POM121C*等^[87-90]. *PAX5*的单倍体剂量不足突变可以协同*STAT5*, *JAK1/3*等功能获得性突变加速B-ALL的疾病进程, 作为一种单倍体剂量不足的抑癌基因参与B-ALL的发生^[4,91,92], 然而*PAX5*缺失的功能在*PAX5*融合的伙伴基因不同的情况下略有不同. 例如, *PAX5-ETV6*融合突变需要*CDKN2A/B*缺失突变的情况下可以激活细胞pre-BCR或者迁移, 黏附等信号活性, 作为促癌蛋白诱导小鼠发病, 然而*PAX5-FOXP1*并无这么强大的驱动作用^[93]; 于此相反, *PAX5-PML*单独存在即可使小鼠诱发B-ALL^[94]. 虽然关于*PAX5*突变的报道的体外细胞实验数据陆续发表, 但是关于其抑癌基因和癌基因功能的体内机制还未得到深入的阐述.

*EBF1*是一种参与调控B细胞特异基因表达的转录调控因子, 它的表达可以使*PAX5*的启动子去甲基化, 与*PAX5*一同参与B细胞的成熟分化^[95]. *EBF1*还可以与转录共激活因子CBP结合, 抑制CBP的赖氨酸乙酰转移酶活性^[96]. 当*EBF1*缺失突变后可以导致相应基因异常的甲基化和乙酰化水平, 加速B-ALL事件发生. *EBF1*的缺失突变在之前提到的融合基因阳性B-ALL患者中很常见, 有研究发现, *EBF1*或者*PAX5*的单倍体剂量不足在起始B-ALL发生过程中发挥非常重要的作用^[92]. 数据显示, *EBF1*可以特异性地抑制*GATA3*的表达, *GATA3*可以通过*STAT4*激活*JAK/STAT*参与B-ALL的发生^[97,98]. *EBF1*还可以被*Zfp521*抑制, 从而使B细胞发育分化受阻^[99,100]. 除了发育和细胞增殖事件受到调控外, *EBF1*的单倍体剂量不足也倾向于和抑癌基因*BTG1*缺失突变协同出现^[101], 驱动B-ALL事件快速发生.

*IKZF1*是一种锌指转录因子家族成员, 通过募集*SIN3A*, *Mi-2b/NuRD*, *CtIP*, *CtBP*等组蛋白去乙酰化酶

图1 B细胞不同发育时期所特有的转录调控因子和信号分子

该模式图展示了B细胞发育相关的关键转录调控因子以及信号分子pre-BCR, BCR等的表达模式; 以及相关转录因子突变导致B细胞分化阻滞时, 白血病克隆表面标志分子的表达水平

共抑制因子, 抑制靶基因的转录; 也可以通过募集BRG1和Cdk9激活靶基因的转录^[102]. 由于IKZF1拥有如此强大复杂的互作关系, 因此IKZF1在所有的淋系分化调控中必不可少. 其由于点突变、片段缺失、基因易位导致的功能缺失在15%的B-ALL患者中被发现^[29,103]. *IKZF1*作为一种抑癌基因突变常和*CDKN2A*缺失突变协同出现, 但是发现单独的*IKZF1*突变似乎不足以阻碍B细胞分化, 需要*PAX5*, *EBF1*, *ETV6*等突变的协助^[104], 或者是通过调控其他的信号通路实现B细胞分化受阻. 例如, IKZF1可以通过促进*CTNND1*和*CD34*的表达阻碍B-ALL的发生^[105,106]. 临床还发现, *ERG* (ETS转录因子家族成员)缺失突变可以中和*IKZF1*突变导致的B-ALL发生, 可以作为B-ALL风险分级和预后良好的一个指标^[58,107].

BCL6 (B cell lymphoma/leukemia gene 6)作为一种锌指转录因子抑制因子, 在B细胞发育分化的起始阶段高表达, 同时也参与细胞周期和凋亡的调控过程. 在B-ALL患者中发现, E2A-PBX1能直接调节*BCL6*的mRNA和蛋白水平, 使其维持在较高的表达水平, 在此过程中, HSC发育调控相关的*LMO2*也同时升高^[108]. 敲除*E2A-PBX1*细胞中的*BCL6*基因后, 细胞周期相关基因*RB1/CDKN1A/CDKN1B*和*CDKN2A*的表达水平下降, 还发现*BCL6*可以与pre-BCR及其下游分子BLK, BANK1, SYK等结合, 促进pre-BCR信号活性和细胞增殖和存活事件. 此外, pre-BCR的抑制剂也可以降低

*BCL6*的表达水平, 选择性地杀伤pre-BCR阳性细胞^[109]. 此外, *BCL6*抑制p53的作用可以被BACH2 (basic leucine zipper transcription factor 2)所逆转, 而且有证据显示, BACH2可以受到PAX5的特异调节, 参与清除未发生V_H-DJ_H重排的pre-B细胞, 显示出抑癌基因的潜质^[110,111]. 由此可见, PAX5-BACH2-BCL6-p53信号轴在B-ALL发生中的关键调控作用. 虽然B细胞发育基因突变在B-ALL发生过程中占有重要作用, 但是其单独存在只是B-ALL发生的必要条件. 研究发现, *CRLF2*过表达(*IGH-CRLF2*或者*P2RY8-CRLF2*融合所致)和JAK激活能协同IKZF1促进B-ALL的发生^[112], 提示B-ALL发生是一个多基因协同突变的遗传异质性肿瘤疾病.

由此可知, 每种B细胞发育调控相关的调控因子通过各自的方式协同起始融合基因突变, 使得B细胞分化受阻, 恶性增殖, 凋亡受限等事件加速出现, 这提示, B细胞发育分化相关调控因子的突变可以作为B-ALL预后不佳的有力证据.

(2) 细胞周期和凋亡相关基因突变. 细胞周期和凋亡调控相关基因在维持细胞自我更新和生存过程中发挥不可替代的作用, 当B祖细胞分裂活动加速且凋亡失控发生时, 极易诱发B-ALL. 临床数据显示, 参与G₁/S细胞周期调控的*CDKN2A/B*缺失突变等占B-ALL患者的25%左右^[82,113]. *CDKN2A/B*突变在*BCR-ABL1*和*TCF3-PBX1*亚型的B-ALL中较高, 在*ETV6-RUNX1*,

*MLL*融合和Hyperdiploidy中较少见^[114]。控制细胞周期和凋亡的*RB1*和*p53*突变在B-ALL中很少,但是相关成员涉及B-ALL的发生^[2,115]。12号染色体长臂微小缺失可以导致*BTG1*突变占B-ALL的10%左右^[4,116],*BTG1*的突变常与*CDKN2A/B*突变相关联^[117],在正常情况下*BTG1*作为一个调控B细胞增殖的负调控因子存在,其缺失突变有利于白血病细胞的恶性增殖。在*E2A-PBX1*复发患者中发现*CDKN2A*和*PAX5*同时缺失突变^[118],提示细胞周期和细胞发育相关基因的协同突变模式或许是促使*E2A-PBX1*阳性B-ALL病程恶化的关键。研究发现,*CDKN2A*上游的BMI1具有增强HSC自我更新的能力,而BMI1能被*E2A-PBX1*特异性激活,BMI1又是INK4A-ARF转录过程的抑制剂,因此*E2A-PBX1*-BMI1-*CDKN2A*信号通路调控机制可能是B-ALL病程进展的重要因素^[119]。与BMI1调控相关的p38 α / β 磷酸化状态在含有*E2A-PBX1*突变的细胞系697中具有促进细胞增殖和存活的功能。当用小分子抑制剂或者敲降方式抑制p38 α / β 活性或者表达时,会进一步延迟白血病发生过程^[120]。2013年,有学者发现TCF3/*E2A*可以通过调控p21:PUMA的表达比例参与细胞周期和凋亡状态^[121],提示细胞周期与凋亡基因突变在*TCF3*相关融合基因阳性B-ALL中具有重要的协同作用。

细胞周期调控相关的Aurora激酶(AURK)家族成员A和B参与正常细胞内中心体成熟、纺锤体组装和染色体分离,在细胞周期调控方面具有重要的作用。*E2A-PBX1*阳性的B-ALL患者中也发现*AURKB*异常高表达,当抑制*AURKB*表达后发现白血病细胞中出现抗增殖和抗凋亡现象,且发现*AURKB*抑制剂能有效抑制此类患者白血病细胞的无限扩增^[122]。*Survivin*作为另一种凋亡蛋白的抑制因子和染色体运输复合体的成员,经常在血液肿瘤和复发的ALL中过度表达,当沉默t(1;19)染色体易位的患者细胞中*Survivin*的表达后白血病细胞的生存能力急剧下降,而*Survivin*抑制细胞生存可能是通过p53介导的细胞凋亡途径实现的,因为这种凋亡过程可在p53抑制状态下被逆转^[123]。综合以上,可以推测细胞周期和凋亡相关基因的缺失突变可能是通过快速累积始发突变所引起的其他基因突变,细胞不能及时修复多种基因损伤进而使亚健康状态的细胞快速进入恶性增殖模式。

(3) 细胞自噬相关基因突变。自噬是细胞内一种包含溶酶体降解、蛋白以及细胞器回收的重要降解途

径,在生理条件下需要基本的表达水平来维持机体HSC的静息、自我更新和分化状态等^[124-126]。机体细胞DNA损伤加重时,其清除降解功能加强。当机体遭遇应激反应和化疗时,自噬是正常细胞和癌细胞存活的一种机制,且在不同的血液肿瘤类型中参与肿瘤耐药和肿瘤抑制两个相反过程^[127]。例如,用酪氨酸激酶抑制剂等化疗药物激活自噬后会引发*BCR-ABL1*阳性患者耐药和复发,因此抗自噬成为该类疾病治疗的关键。最近文献报道,自噬相关基因*Beclin1*,*Atg7*,*Atg5*,*LC3*,*p62*等在B-ALL细胞中表达水平降低,当用雷帕霉素诱导自噬活化后,*E2A-PBX1*蛋白表达降低,且发现,自噬能特异性降低聚合酶DNA pol δ 1和RNA pol II活性,进而抑制白血病细胞增殖,同时细胞周期也发生阻滞^[128]。但是自噬是否需要其他调节蛋白的参与影响疾病进程?研究发现,*E2A-PBX1*蛋白分布在自噬体内的自噬分子LC3附近和泛素化相关分子周围,且自噬和泛素化抑制剂能阻止*E2A-PBX1*蛋白和相关蛋白靠近,提示自噬和泛素化有协同降低*E2A-PBX1*蛋白的作用^[129]。随后发现,针对自噬相关抑癌基因特征设计出来的抗肿瘤药物Bortezomib,可以在破坏Bcl-2/*Beclin-1*复合体的同时促进*Beclin-1*/PI3KC3复合体形成,进而抑制自噬事件的发生,最终达到杀伤B-ALL细胞的目的^[130],这种治疗策略很快地也在糖皮质激素耐药的B-ALL细胞中也得到充分的诠释^[131]。自噬相关突变在B-ALL作用显著,但是自噬活动在有附加基因突变(如聚合酶和泛素化酶等基因突变导致的自身蛋白失活或持续激活等)的状态下是否还能抑制细胞的恶性转化还不清楚。

(4) 细胞增殖存活相关信号通路基因突变。造血干细胞在稳态造血体系中处于最顶端位置,当其向B细胞分化进程中自我更新和分化状态失去平衡后很快就会转化为白血病细胞,而各种细胞信号通路的异常可能是影响这一过程的关键。研究发现,*RAS*信号通路突变频率(*NRAS*,*KRAS*,*NFI*)在*MLL*和高染色体突变的B-ALL患者中较高^[132,133],同时也在6%的Ph like ALL患者中被检测到^[134]。在融合基因起始促癌发生的状态下,信号通路之间的协同必定发挥着至关重要的作用,参与白血病细胞的存活、增殖、分化等活动。例如,HSC自我更新相关的蛋白HOXA9可以通过激活FLT3, Pdgfr δ 信号通路赋予*E2A-PBX1*阳性B-ALL细胞异常增殖和B细胞发育分化阻滞的能力^[41]。有证据显

示, E2A-PBX1融合蛋白的N端PCET结构域能和CBP/p300的KIX结构域直接互作, 干扰B细胞的发育和增殖事件^[135,136]. 而CBP/p300又和Wnt信号成员catenin存在互作, 干扰细胞的分化状态, 这一过程可被CBP的小分子特异性抑制剂ICG-001所抑制, 使此类患者对传统的化疗药物敏感^[137]. 最近发现, PKC β 抑制剂ENZ介导的 β -catenin过量累积导致下游*c-Myc*表达降低, *c-Jun*表达升高, 进而导致抑癌基因*p73*稳定发挥作用, 最终导致细胞凋亡和细胞周期阻滞^[138]. 因为PKC β 有介导pre-BCR信号通路的作用, 这也提示, pre-BCR信号通路和wnt信号通路之间可能存在crosstalk作用. 2017年, 有学者首次发现SERCA依赖的信号通路(Sarco/endoplasmic reticulum calcium ATPases)和B细胞分化阻滞之间存在crosstalk作用^[139]. 该研究指出, 当使用Phorbol ester激活PKC激酶活性后, 会使得E2A-PBX1阳性细胞系中SERCA3依赖的钙泵激活, 而SERCA3的表达升高却是成熟B细胞的标志, 提示SERCA3可能具有抑癌基因的功能. 近期发现E2A-PBX1小鼠和患者中如果存在JAK/STAT (*Jak1*, *Jak3*, *Ptpn11*, *IL7r*), RAS/MAPK (*Nras*和*Kras*)异常激活突变, 其发病周期也会明显缩短^[140,141]. 综合来看, 单一的信号通路可能不足以引起融合基因突变所引起的细胞恶性增殖. 多种细胞信号之间的特异互作可能是融合基因突变引起和维持白血病细胞过度增殖、细胞凋亡失控的必要条件. 那么针对不同信号通路的靶向药物的协同使用或许是避免B-ALL化疗药物大剂量使用产生毒性副作用的关键.

(5) 表观遗传调控相关基因突变. 众所周知, 表观遗传修饰相关的酶可以“书写”和“擦除”细胞内相关基因的修饰水平, 参与正常的胚胎发育和造血过程. 常见的表观遗传修饰调控包括DNA甲基化、组蛋白乙酰化以及microRNA介导的基因转录调控等. 那么表观遗传修饰相关协同基因突变在B-ALL表达过程中具有什么作用? 全基因组甲基化分析显示, 临床上4种常见的B-ALL亚型中DNA甲基化模式有所差异, *ETV6-RUNX1*患者存在许多高甲基化修饰, 而*TCF3-PBX1*和超二倍体患者甲基化程度较低^[142]. 或许可以根据患者甲基化程度进行风险分级, 判断预后等状态. 有学者发现, E2A-PBX1突变的B-ALL患者中*p73*和*MGMT*高度甲基化, 基因转录水平降低, 导致基因沉默发生, 而*E-cadherin*, *DAP-kinase*, *p16*, *p15*, *p14*未发现甲基化, 但缺乏深入的功能研究^[143]. 那么DNA甲基化在B-ALL发

展的调控机制如何? 随后人们发现E2A-PBX1突变的患者中甲基化程度较低的基因异常高表达, 可能是与E2A, PBX1, p300的DNA结合区域结合引起的^[144,145]. H3K79甲基转移酶Dot1活性缺失后能减弱MLL融合基因导致的白血病细胞存活和克隆形成潜能, 但在E2A-PBX1阳性ALL中未能发现.

组蛋白乙酰转移酶HATs和组蛋白去乙酰转移酶HDACs可以通过改变染色体的疏松结构参与白血病的发生. 例如, 通过shRNA手段选择性地降低HDAC1和HDAC2的表达可以抑制B-ALL细胞系的增殖^[146]; 与之相反, HDAC7在B-ALL中表达降低, 通过抑制凋亡, 促进原癌基因*c-myc*的表达而促进B-ALL的发生^[147]. 蛋白乙酰化修饰中的*p300/CBP*, *GCN5*和*PCAF*在基因转录后修饰过程中同样发挥着重要的作用, 这种调控模式主要通过组蛋白乙酰化打开凝集的染色质实现沉默基因的再次表达, 参与细胞分化和肿瘤发生过程. 有实验首次证实, E2A-PBX1融合蛋白的AD2与CBP/p300的KIX结构域直接互作, 可以明显提高CBP/p300固有的乙酰转移酶HAT活性, 从而导致E2A-PBX1融合蛋白的靶基因转录水平受到干预, 影响细胞增殖和分化事件^[136,148]. 另外, GCN5通过形成的SPT3-TAFII31-GCN5L复合体乙酰化E2A-PBX1融合蛋白的E2A区域, 从而增加其在白血病细胞中的稳定性, 阻止E2A-PBX1蛋白发生降解, 而泛素连接酶HDM2通过与PBX1互作, 具有降解E2A-PBX1蛋白的作用. GCN5的这种特殊效应通过GCN5蛋白抑制剂MB-3显著降低E2A-PBX1蛋白在白血病细胞内的表达水平所验证^[149]. 以上提示, 乙酰化修饰在维持融合基因表达水平的重要作用, 同时也可以看出表观遗传调控通过间接的方式可以参与B-ALL的发生发展.

microRNA是一种21个核苷酸长度的小片段, 能和特异的靶基因mRNA的3'端非编码区结合, 抑制其转录活性, 参与机体造血、细胞分化和凋亡等重要事件, 基于此, miRNA在B-ALL的作用也逐渐受到人们的关注. 临床数据显示, B-ALL患者中*miR-222*, *miR-339*, *miR-142-3p*等癌基因表达升高, 相反, *hsa-miR-451*, *hsa-miR-373*, *miR-196b*, *miR-124a*, *miR-143*, *miR-509*等抑癌基因表达降低^[150-154]. 其中, *miR-509*可以通过抑制RAB5C阻碍B-ALL细胞的恶性增殖. 又如*miRNA-126*在HSC中的表达可以展现出癌基因的功能, 诱导B-ALL表型CD19⁺B220⁺IgM⁻细胞群体的异常扩增, 当撤

销*miRNA-126*的表达后B细胞发育相关基因(*Myb*, *Mafk*, *Ebf1*, *Irf1/3*, *Pbx1*, *V(D)J*重排相关基因(*Rag1/2*, *Lig4*)以及DNA修复和抑制细胞周期调节因子相关基因(*Rbl*, *Cdkn1b*, *Cdkn2c*, *Chek1/Clspn*, *E2f7*, *Pml*)表达异常升高^[155]。有学者通过细胞功能实验发现, 具有癌基因功能的*miRNA-126* (*TEL-AML1*患者)表达水平升高不能改变*E2A*突变细胞系的细胞周期和凋亡状态, 可能*miR-126*需要在特定的组织中才具有促癌基因的作用^[156,157]。最近有学者比较系统地分析了*miRNA*主要通过靶向于PI3K-Akt, Jak-STAT, Ras和细胞周期相关信号通路参与B-ALL的发生发展^[158]。此外, 还有近年来发现的lncRNA可以通过结合*E2A*调节HSC的自我更新和分化功能, 在*E2A-PBX1*患者中也发现lncRNA中的*BALR-2* (B-ALL associated long RNAs 2)表达升高可以促进细胞的增殖和对prednisone的耐药性^[159,160]。综上可知, 非编码RNA可以分为癌基因和抑癌基因两类参与B-ALL发生, 也基于RNA自身的结构特点和代谢特点, 可以为临床相关患者设计出符合自身的特异性小分子抑制剂或者特异互补片段, 干扰相关非编码RNA导致的疾病过程。

1.3 B-ALL相关基因突变与LSC

LSC由于自身存在的干性重建能力、自我更新潜力、静息状态和药物耐受等特点成为近年来人们关注的焦点, 目前研究LSC的起源主要是基于含有各种基因突变的起始细胞HSC/HPC等是否能完成白血病恶性转化过程来判读其LSC起源问题。起初对LSC的假说多集中在AML模型中, 认为LSC起源于发生恶性转化的HSC或者HPC^[161]。近年来LSC所具有的功能在B-ALL中也逐渐被报道^[162-164], 例如, *TEL-AML1*融合蛋白可以赋予人HSCs更强的自我更新能力, 提示LSC可能来源于含有起始突变基因的HSC^[165], 这一假设在BCR-ABL^{p210}以及N-myc诱导的HSC最终发生B-ALL模型中也得到证实^[166,167]。然而, 有数据对上述观点提出质疑, *TEL-AML1*突变可以赋予胎鼠Pro-B细胞(B220⁺ c-kit⁺)自我更新特性^[168], 这种不同的结果可能是由于LSC在人和鼠不同的微环境下向B细胞发育的差异造成的。但是近年来发现, MLL-AF4阳性的Pro-B细胞中含有淋系髓系标记物, 提示LSC可能起源于淋系和髓系祖细胞^[169]。由于LSC起源存在争议, 所以其标记分子也很难确定, 但是最近有证据显示, BCR-

ABL诱导的B-ALL中, LSC的表型为CD34⁺ CD38⁻ CD58⁻, 这一结果与人源*TEL-AML1*突变发现的LSC具有相似性^[170]。

虽然LSC起源和标记存在争议, 但是可以从HSCs调控机制的角度去探索LSC的细胞活动, 进而为临床治疗复发B-ALL提供思路。例如有证据显示, 白血病发生相关的BMI1活性升高时, 可以通过表观遗传调控方式抑制EBF1和PAX5的表达, 从而维持HSCs的干细胞活性^[171], 提示BMI1在维持LSC自我更新能力方面可能具有重要作用。但是在杀伤LSC的同时如何保证正常的HSCs免受伤害还存在一定挑战。由于B-ALL是一种范围较广的遗传异质性恶性肿瘤, 因此也预示着LSC起源问题在不同基因突变的B-ALL有所差异, 这对于寻找LSC的确切起源和分子标记形成很大阻碍, 需要比较可靠的小鼠模型来验证LSC的起始演变过程。目前关于LSC的发现也是基于将起始突变细胞移植到小鼠体内, 那么小鼠的白血病重建是否可以真实地反映人B-ALL的表型复发进程还有待探讨。虽然B-ALL中LSC确定的分子标记还存在争议, 但是LSC在B-ALL复发过程的作用举足轻重, 因此更加确定清晰的LSC起源和白血病克隆演变过程对于提高复发性B-ALL的治愈率非常重要。

那么这些具有不同细胞起源的白血病克隆细胞如何演变呢? 研究显示, 具有乙酰化转移酶活性和泛素连接酶活性的*CREBBP*缺失突变在20%的复发的患者中很显著, 且在白血病克隆主群和初诊时的某一克隆细胞群存在。缺失的突变主要集中在HAT (histone acetyl transferase)结构域, 破坏了组蛋白靶点的乙酰化水平^[172]。也有类似的数据显示, *p53*和*NT5C2*突变均是在复发时获得^[173,174]。*p53*的突变具有偏向性, 主要集中在除ETV6-RUNX1和BCR-ABL1外的相关B-ALL中, 且预后较差^[175]。通过高通量基因芯片分析复发患者的克隆演变历程, 发现复发时白血病克隆细胞的基因突变除了和初诊时相同或者在初诊基础上获得新的基因突变外, 有些复发克隆细胞中缺失了初诊时的DNA CNAs (DNA copy number alterations), 并且获得新的基因突变。同时发现初诊和复发时的都可以检测到相同的基因突变, 提示复发克隆细胞可能来自初诊时的少数前体白血病克隆细胞。另外也发现, 复发克隆完全缺失初诊时的基因突变模式, 提示复发克隆可能来自更加原始的正常HSPC基因突变引起的白血病(图

2)^[113,174,176-180]. 从白血病复发克隆演变过程可以猜测, 可能复发时的基因突变模式改变了机体微环境状态, 使得白血病细胞易于存活, 逃避免疫杀伤和化疗作用, 同时也抑制机体正常的造血能力.

2 B-ALL复发难治的主要因素

虽然根据B-ALL患者中枢神经系统浸润程度、年龄、白细胞计数、微小残留病(micro residual disease, MRD)水平、细胞遗传分析等手段可以进行有效的风险分级从而施用不同强度的化疗方案, 但是这些指标并不能总是将所有高危患者甄别出来并得到妥善的治愈. 随着二代测序技术的应用, 越来越多的与B-ALL复发难治相关的主要基因突变以及基因突变的组合模式被揭示出来. (i) 特定协同基因的突变模式导致患者复发可能和某些B-ALL亚型患者密切相关. 通过外显子测序发现*NT5C2*, *CREBBP*, *WHSC1*, *p53*, *USH2A*, *NRAS*, *IKZF1*等突变和复发风险相关^[174]. 例如, *IGH*与*ID4* (inhibitor of DNA binding 4), *EPOR*, *BCL2*, *LHX4*, *IL-3*, *CRLF2*等融合形成融合蛋白^[181-185]. 其突变形式在成人B-ALL中较常见, 且预后较差^[184]. 又如*iAMP21*患者中可以发现B细胞发育相关的*RUNX1*的功能获得性突变和复发相关^[186]. 另外B细胞发育相关的基因

*IKZF1*也与预后较差极为相关^[187], 但是*IKZF1*的突变效应可以被*ERG*缺失突变所逆转^[58]. *CREBBP*的突变多集中在hyperdiploid表型的B-ALL中^[188], 且发现HAT结构域突变使得白血病细胞对糖皮质激素诱导的细胞杀伤作用减弱, 或许是糖皮质激素耐药的机制. (ii) 不同基因突变之间的协同组合模式可能也是B-ALL复发的关键. 例如, *CD200/BTLA*缺失突变联合*IKZF1*缺失或者*iAMP21*表型突变可使B-ALL患者更容易复发^[189], *CDKN2A/B*与*IKZF1*同时缺陷也预示患者预后较差^[8].

基于对复发因素的探索, 临床相关治疗策略也得到一定启示. 例如有数据显示, mTOR信号通路受到抑制有升高Ph⁺和Ph like患者白血病细胞数的风险, 提示选用化疗方案时应避免mTOR或者上游信号受到抑制^[190]. 再如B细胞发育相关的PI3K δ (phosphatidylinositol 3-kinase δ , 是pre-BCR和IL7R信号的关键成员)被抑制后促进了糖皮质激素介导的白血病细胞杀伤效果^[191]. 另外发现, 和泛素化相关的USP9X功能失活可以抑制JAK2活性从而促进白血病细胞的存活, 颠覆了人们对JAK2信号的认识^[192]. 最近发现, 黏附相关的Fat1和免疫调控相关的Toll样受体TLR在B-ALL的复发过程中具有重要作用, 但是发病机制不详^[193-195]. 虽然BCR-ABL患者的治愈率得益于酪氨酸激酶抑制剂, 但是最近发现, BCR-ABL患者中发现TKI治疗后的复

图2 B-ALL患者从初诊到复发过程中的白血病克隆演变模式图

该图展示了复发时的白血病克隆所包含的基因突变种类与初诊时白血病克隆的基因突变种类之间的演化关系, 提示不同致病条件下, 白血病干细胞可能存在不同的细胞起源

发患者BCR-ABL中的酪氨酸激酶结构域含有E459K, E255K, V299L点突变, 限制了TKI的靶向作用^[196]。由此可知, 复发性B-ALL的治疗方案因人而异, 且其治愈率提升的每一步都离不开对B-ALL发病机制的深入探索。

3 B-ALL相关小鼠模型的应用

B-ALL的发生和克隆演变涉及很多相关基因的异常表达, 尽管临床上每一种基因突变在细胞系中都能得到快速的验证, 但是体外实验并不能完整地模拟体内的复杂环境变化, 得出的结论未必真实。因此一个可以真实反映临床B-ALL发病进展的理想动物模型是理解其致病机制和探索治疗手段的有力工具。

3.1 经典的B-ALL小鼠模型

根据实验目的不同目前的小鼠模型可以分为两种: 免疫缺陷小鼠和病毒感染后异体移植小鼠。虽然免疫缺陷小鼠可以用于研究白血病细胞的起源, 但是受体小鼠的免疫功能受损, 以及自身微环境不同于人的微环境, 势必影响LSC的克隆演变过程。第二种移植小鼠在不同实验室的照射剂量不同也会对LSC或者移植的供体细胞生长产生影响, 以及病毒本身随机整合引入的突变, 从而导致实验重复性和一致性较差。因此, 结合B-ALL遗传异质性的特点和研究问题的角度等, 设计出特定基因突变组合或者可诱导的基因突变相关遗传修饰小鼠是研究B-ALL发病的理想手段。

3.2 特定启动子下携带融合基因的遗传修饰小鼠模型

自从融合基因被发现以来, 人们便逐渐开始了在小鼠特定启动子表达下的融合基因小鼠模型尝试。例如有学者证实BCR-ABL^{p190}融合蛋白在金属硫蛋白启动子下可以使小鼠在几周内死于淋系或者髓系白血病发生^[197], 在BCR启动子下却可以诱导发生B-ALL^[198], 但是持续地表达BCR-ABL^{p190}可以导致F₁小鼠胚胎致死, 有实验还证实BCR-ABL^{p190}本身具有ALL发病倾向, 并不需要BCR特异启动子参与^[198]。又如TEL-AML1在IGH增强子和启动子下诱导的小鼠不发生任何疾病^[73]。因此, 对应的融合基因与合适的启动子可能是B-ALL发生的关键。除了启动子外, 也有部分学者认为,

合适的协同基因突变结合特定启动子表达下的融合基因可以诱发B-ALL, 例如, BCR-ABL^{p210}一般认为很难诱发B-ALL, 但是有证据显示, BCR-ABL^{p210}与p16^{INK4A}/p19^{ARF}缺失可以诱发B-ALL^[167]。也可以通过调整融合基因的融合片段策略实现定向B-ALL发病, 例如, MLL融合相关的小鼠模型常导致AML和非B-ALL的产生极大地限制MLL融合相关的B-ALL发生。以往的MLL-AF4融合基因病毒滴度较低很难感染靶细胞发生B-ALL, 但是最近有研究发现, 可以将人的MLL与小鼠的AF4融合产生滴度较高的病毒, 感染小鼠HSPC, 导致B-ALL的发生^[56]。

可诱导的遗传修饰小鼠模型也在研究B-ALL的发生机制中广泛应用。例如, 在四环素诱导系统启动子下表达的BCR-ABL^{p190}可以使小鼠发病, 当撤去诱导条件后因为BCR-ABL^{p190}导致肿瘤起始细胞的表现遗传修饰相关基因发生改变, 使得B-ALL的表型持续存在, 符合临床相关克隆演变过程^[199]。又如将Mx1Cre诱导下Stat5表达的小鼠骨髓细胞(5-fluorouracil预处理过)感染BCR-ABL^{p210}病毒后发现, Stat5b的表达升高具有维持BCR-ABL^{p210}诱导的CML表型的作用^[200]。利用这种可诱导的条件还可以研究其他可变因素在B-ALL发生机制, 例如, 上述可诱导的BCR-ABL^{p190}在研究年龄对B-ALL的影响时发现, 年老小鼠比年轻小鼠更容易发病^[201]。在Cd19, Mb1, Mx1启动子下的Cre小鼠与E2A-PBX1小鼠杂交后, B-ALL发生率达5%~50%左右^[140]。又如Mx-Cre小鼠与相应的E2A-HLF小鼠杂交, 得到Cre诱导的E2A-HLF小鼠, 通过病毒随机整合导致的二次基因突变发现, 少部分小鼠被诱导为B-ALL, 且发现Gfi1, Ikaros, Zfp521 (zinc-finger protein 521)发生过表达, 而Zfp521被证实在人E2A-HLF阳性细胞系中过表达^[202]。又如将LOXP位点引入MLL和ENL所在的9和17号染色体上, 再用Lmo2-Cre去诱导染色体易位导致的融合基因表达, 但是此小鼠在数月内发生MPD样髓系白血病(myeloproliferative-disease-like myeloid leukemia)^[203]。相应的MLL-AF4小鼠与pan-haematopoietic Mx1-Cre小鼠杂交后, 部分小鼠显示白血病细胞表型和pre-B细胞类似^[204]。

3.3 利用协同基因策略构建的小鼠模型

由于B-ALL是一种多基因突变的疾病, 因此在研

究基因协同突变致病机制时, 经常会将融合基因小鼠和协同基因突变小鼠(*PAX5*, *IKZF1*, *Stat5*)杂交, 获取两种基因突变模式小鼠, 此方法较为巧妙地避免了病毒感染对细胞造成的随机整合突变. 例如, *BCR-ABL*^{p190}转基因小鼠与单倍体剂量不足的*IKZF1*小鼠杂交可以证明*IKZF1*可以加速*BCR-ABL*始发的B-ALL发生进程^[205]. 例如, *p19Arf*^{-/-}小鼠与*Rag1*^{-/-}小鼠杂交得到小鼠诱发B-ALL, 其中Sca-1⁺CD19⁺细胞群含有受Notch信号调控的LSC^[206]. 在研究基因突变协同E2A-PBX1诱导的B-ALL中, 可以事先将E2A-PBX1小鼠和*CD3ε*^{-/-}小鼠杂交, 在通过外部施进癌变的因素的方式研究*Hoxa*协同E2A-PBX1的发病机制^[207]. 又如*TEL-AML1*小鼠和*T2Onc*转座子小鼠杂家得到的小鼠是目前*TEL-AML1*可以诱发B-ALL的唯一模型, 但是这种模型也会诱发AML和T-ALL^[208]. 虽然可将融合基因病毒感染在含有协同基因突变的小鼠细胞内, 然后移植到受体小鼠体内, 评估协同基因在维持B-ALL表型的作用. 例如, *BCR-ABL*病毒感染*Arf*^{-/-}小鼠来源的骨髓细胞诱发白血病发生^[209], 或者AID (activation-induced cytidine deaminase)和C/EBPβ在促进*BCR-ABL*致病的机理^[210,211]. 但是此类方式得到结果可能存在潜在的不确定性.

随着对协同基因的深入研究, 同一种基因突变不同的小鼠模型也逐渐受到人们的关注, 这为研究相同基因不同突变模式导致的B-ALL患者的个体化治疗提供了很好的研究平台. 目前协同基因功能缺失或获得的转基因小鼠模型目前还很少见, 相对开发较早的是*PAX5*功能缺失小鼠模型. 第1种*PAX5*小鼠模型是在*CD19*启动子下表达的Cre诱导*PAX5*^{fl/-}突变, 导致外周血B细胞*PAX5*缺失, 产生B细胞淋巴瘤^[212]. 第2种*PAX5*小鼠模型是*PAX5*^{+/-}与*Stat5b-CA*杂交后使B-ALL的发生率由1%提升到100%^[92]. 第3种小鼠*PAX5*小鼠模型是研究*PAX5*^{+/-}小鼠累积二次突变的种类, 研究显示, 切除胸腺的*PAX5*^{+/-}小鼠暴露在ENU (*N*-ethyl-*N*-nitrosourea)或者MMLV(moloney murine leukemia retrovirus)后发现B-ALL表型中含有JAK家族基因激活突变^[91]. 第4种*PAX5*小鼠模型是直接将*PAX5*^{+/-}小鼠从SPF级动物房转移到普通环境遭遇感染后小鼠获得*JAK3*二次基因突变^[213]. 第5种*PAX5*小鼠模型是将人的*ETV6*序列插入到小鼠的*PAX5*位点, 结合*CDKN2A/B*缺

失, 使小鼠发病^[93]. 第6种*PAX5*小鼠模型是将*PAX5*小鼠和*Stat5b-CA*杂交得到*PAX5*^{+/-}×*Stat5b-CA*表型的小鼠, B-ALL发病率由1% (*Stat5b-CA*小鼠)提升的100%^[92]. 虽然以上*PAX5*的小鼠修饰模型能从不同的角度很好地反映临床相关协同基因缺失导致的B-ALL发生, 但是小鼠自身基因突变和人还是存在一点差距, 并且其他大多数协同基因突变还主要是通过病毒感染来实现的, 存在病毒随机整合的问题, 例如, 最近报道的*PAX5-PML*和*N-myc*诱导小鼠B-ALL的发生(表3)^[94,166,214].

目前尽管关于B细胞发育, 细胞周期和增殖相关基因突变的小鼠模型被部分开发出来, 但是总体上发病周期长, 表型微弱或者不能诱发完整的B-ALL小鼠表型, 难以满足临床需要. 小鼠模型中含有可诱导的精准时间点如何与临床上白血病克隆起始和复发演变模式有机地结合起来还不清楚, 将来应用CRSPR/CAS9等精准的基因编辑技术结合细胞特异表达的Cre诱导系统或许可以对临床发现的不同基因突变模式进行贴切的模拟.

4 展望

综合可以看出, B-ALL的发生是一个多基因突变的复杂过程, 涉及早期融合基因突变和后期协同基因突变的综合作用. 尤其重要的是, B细胞发育相关的基因突变在B-ALL的发生过程中扮演着越来越重要的角色. 纵观历史, 人们对于正常造血调控机制和免疫系统调节机制的每一种新发现, 都会带来B-ALL病理机制的深刻认识, 因此对于正常造血系统的发育调控机制的深入且详实可靠的理解必不可少. 尽管近年来通过高通量分析, 二代测序以及B-ALL小鼠模型的应用发现了一些协同基因突变(细胞分化、增殖、凋亡、自噬、表观遗传调控和信号通路的相关基因突变)类型在B-ALL恶性转归和复发过程中具有重要的作用, 也给相应的B-ALL表型提供了有效的治疗策略, 但是很多基因功能和基因与基因之间的协调作用并不清楚, 仍有很多未知领域亟待解决, 需要漫长的时间去逐一验证. 目前认为大多数的白血病细胞起源于pro-B祖细胞, 那么起源于干性更强细胞来源的B-ALL是否具有不同的药物敏感性和复发风险还需要进一步验证. 例如, 还有很多复发难治的B-ALL缺乏精准的诊断标志分子, 给B-ALL的预防和分类带来极大障碍. 目前已

表3 基于B-ALL相关基因突变所构建的小鼠模型分类^{a)}

构建日期	小鼠模型	小鼠表型	额外刺激条件	发病率	PMID号
1990	<i>BCR-ABL</i> ^{p190}	AML/ALL	No	—	2179728
1997	<i>BCR-ABL</i> ^{p190} (bcr ⁻)	B-ALL (B220 ⁺ , Thy-1.1 ⁻ /Thy-1.2 ⁻ , Mac1 ⁻ , Gr-1 ⁻), Sig ⁻)	No	92.5%	9310467
2008	<i>p16INK4A/p19ARF</i> ^{-/-} × <i>BCR-ABL</i> ^{p210}	B-ALL (B220 ⁺ , CD19 ⁺ , IgM ⁻ , A44.1 ⁺)	No	100%	18755985
2010	<i>IKZF1</i> ^{+/-} × <i>BCR-ABL</i> ^{p190}	B-ALL (B220 ⁺ , sIgM ⁻ , CD43 ⁺)	No	100%	20393504
2010	<i>BCR-ABL</i> ^{p210}	CML	Stat5b Cre	0	20201032
2005	<i>E2A-PBX1</i>	B-ALL (B220 ⁺ , CD43 ⁻ , CD19 ⁺ , IgM ⁻)	<i>CD3ε</i> ^{-/-}	40%	15655112
2005	<i>E2A-PBX1</i>	B-ALL (B220 ⁺ , CD43 ⁻ , CD19 ⁺ , IgM ⁻)	<i>CD3ε</i> ^{-/-} 和 MMLV	66%	15655112
2015	<i>Mx1 Cre</i> × <i>E2A-PBX1</i>	B-ALL (B220 ⁺ , CD25 ⁺ , Bp1 ⁺ , CD24 ⁺ , IL-7α ⁺ , CD117 ⁺ , CD79a ⁺)	No	59%	26301816
2015	<i>Cd19 Cre</i> × <i>E2A-PBX1</i>	B-ALL (B220 ⁺ , CD25 ⁺ , Bp1 ⁺ , CD24 ⁺ , IL-7α ⁺ , CD117 ⁺ , CD79a ⁺)	No	7%	26301816
2015	<i>Mb1 Cre</i> × <i>E2A-PBX1</i>	B-ALL (B220 ⁺ , CD25 ⁺ , Bp1 ⁺ , CD24 ⁺ , IL-7α ⁺ , CD117 ⁺ , CD79a ⁺)	No	53%	26301816
2010	<i>Zfp521</i> ^{+/-} × <i>E2A-HLF</i>	B-ALL (B220 ⁺ , Mac1 ⁻)	No	Very low(no data)	20062079
2011	<i>T2Onc</i> ^{+/-} × <i>ETV6-RUNX1</i>	B-ALL (B220 ⁺ CD19 ⁻ , IgM ⁻ , IgD ⁻)或 AML/T-ALL	No	21.1%	21628403
2016	<i>MLL-AF4</i>	B-ALL (CD19 ⁺ , CD45 ⁺ , CD33 ⁺ , CD34 ⁺ , CD10 ⁺)	NSG Mice	100%	27846391
2008	<i>Mx1-Cre</i> × <i>MLL-AF4</i>	B-ALL (B220 ⁺ CD19 ⁺ Mac1 ⁻)	No	28%	18977325
2007	<i>CD19 Cre PAX5</i> ^{fl/-}	Lymphoma (B220 ⁺ , Igμ ⁺ , CD93 ⁺ , CD25 ⁺ , IL-7Rα ⁺ , c-kit ⁺ , Flt3 ⁺ , CD19 ⁻ , IgD ⁻)	No	100%	17851532
2011	<i>Stat5b-CA</i> × <i>PAX5</i> ^{+/-}	B-ALL (AA4.1 ⁺ , IL7R ⁺ , B220 ^{low} , CD19 ⁺ , IgM ⁻ , preBCR ⁻ , IgD ⁻)	No	100%	21606506
2015	<i>PAX5</i> ^{+/-}	B-ALL (B220 ⁺ , CD19 ⁺ , BP1 ⁺ , IgM ⁻)	Thymectomy plus ENU or MMLV	100%	25855603
2015	<i>PAX5</i> ^{+/-}	B-ALL (<i>CD19</i> ^{+/-} , B220 ⁺ , IgM ⁻ , cKit ⁺ , CD25 ⁺)	Common infections	22%	26408659
2016	<i>PAX5-PML</i>	B-ALL (B220 ⁺ , CD43 ⁺)	No	100%	26703467
2017	<i>PAX5-ETV6</i> × <i>CDKN2A/B</i> ^{+/-}	B-ALL (CD93 ⁺ , CD25 ⁺ , IL-7Rα ⁺ , CD19 ⁺ , Igμ ⁺)	No	100%	28219927
2017	<i>PAX5-FOXP1</i> × <i>CDKN2A/B</i> ^{+/-}	Pro-B phenotype (CD19 ⁺ , B220 ⁺ , c-Kit ⁺ , CD2 ⁺ , CD25 ⁺ , IgM ⁻ , IgD ⁻)	No	0	28219927
2011	<i>Ebf1</i> ^{+/-} × <i>Stat5b-CA</i>	B-ALL (AA4.1 ⁺ , IL-7R ⁺ , B220 ⁺ , CD19 ⁺ , IgM ⁻ , IgD ⁻ , pre-BCR ⁻)	No	100%	21606506
2017	<i>N-myc</i>	B-ALL (B220 ⁺ , CD19 ⁺ , Mac1 ⁻ , Gr-1 ⁻ , IgM ⁻ , CD43 ⁺)	No	100%	21986948;27-941793
2011	<i>p19Arf</i> ^{-/-} × <i>Rag1</i> ^{-/-}	B-ALL (CD19 ⁺ , IgM ⁻ , IL7Rα ⁺ , CD25 ⁺ , BP1 ⁺ , CD24 ⁺ , B220 ⁺)	No	65%	21622646

a) CA: constitutively active; ×: 两种小鼠之间需要交配; *: +或者-; bcr⁻: bcr启动子是非必需的

知的复发型B-ALL发病分子机制还不清楚, 主要受限于目前所应用的小鼠模型, 还不能满足人们对于各型B-ALL发病机制的深入探索. 另外由于常见的融合基因的融合位点存在差异, 导致融合蛋白的空间三维结构存在随机异质性, 对于解析其空间结构也产生一定程度影响. 近年来发现的ESCRT (endosomal sorting complexes required for transport)成员在造血分化中的调节作用或许能从新的角度促使人们理解B-ALL发生

和复发的真实过程. B-ALL患者表观遗传修饰中的DNA甲基化, 组蛋白乙酰化及去乙酰化, microRNA等调节机制还停留在起步阶段, 还有待进一步深入挖掘. 其他肿瘤中发挥重要作用的RNA输出(基因转录和翻译之外的基因表达调控方式)在B-ALL发生过程中有何影响也不清楚. 相信通过对B-ALL复发机制的深入了解, 可以使研究者们找到与诊断B-ALL复发相关的分子以及相应的精准治疗策略.

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The mechanism of B cell acute lymphoblastic leukemia development

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B cell acute lymphoblastic leukemia (B-ALL) is a common malignant disease in children. Gene mutations in transcriptional factors and signal transduction molecules result in differentiation block and abnormal proliferation of B lymphoid progenitor cells. Children with B-ALL generally have a good prognosis, but about 20% of the pediatric patients and 60% adult patients still suffer from the high risk of relapse. Better understanding of the mechanism of leukemogenesis leads to significant improvement in treatment of ALL patients. Gene expression analysis and whole genome sequencing technologies have identified a number of novel gene mutations, but the roles of those mutations in leukemogenesis remain unclear. This review mainly summarizes the so far identified cooperating gene mutations related to cell development and differentiation, epigenetic regulation, cell cycle and apoptosis, signal pathway, non-coding RNA, and leukemia clone evolution, some of which may be tightly associated with the relapse of B-ALL. In addition, we also review mouse models of B-ALL which may be useful for study of pathogenesis, risk classification, and design of future therapy.

B-ALL, fusion gene, cooperative gene, leukemia stem cells, mouse model

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