

斑马鱼血液疾病模型及应用

郭佳妮, 刘帆, 王璐

中国医学科学院血液病医院(中国医学科学院血液学研究所), 实验血液学国家重点实验室, 国家血液系统疾病临床医学研究中心, 天津 300020

摘要: 血液发育是一个复杂有序且保守的过程, 由多种转录因子和信号通路协同调控, 任何环节的失调都可能引起血液系统发育或功能缺陷, 导致血液疾病的发生。斑马鱼(*Danio rerio*)造血过程及分子调控机制与哺乳动物高度保守。应用斑马鱼模拟致病因子的异常变化构建相关血液疾病模型, 为探究疾病发生机制、肿瘤发生发展可视化研究及高通量化学筛选提供了有力的工具。本文概述了斑马鱼血液疾病模型及其应用, 这些疾病模型不仅有助于完善对血液系统病理生理学、血液疾病发生分子机制的认识, 也为临床相关恶性血液疾病的治疗提供了新思路。

关键词: 斑马鱼; 血液疾病; 动物模型; 化学药物筛选

Zebrafish blood disease models and applications

Jiani Guo, Fan Liu, Lu Wang

State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China

Abstract: Hematopoiesis is a complex, orderly and conserved developmental process, coordinated by multiple factors including transcription factors and signaling pathways. Dysregulation of any of these factors may cause developmental or functional defects in the blood system, leading to the pathogenesis of blood diseases. Zebrafish hematopoiesis and the underlying molecular mechanisms are highly conserved with those in mammals. The use of zebrafish to recapitulate abnormal changes in pathogenic factors can build models of related blood diseases, thus providing powerful tools for exploring the molecular mechanisms of pathogenesis and progression, visualization of tumorigenesis and high-throughput chemical screening. In this review, we summarize the zebrafish models of blood diseases and their applications. These disease models not only help to improve our understanding of the pathophysiology of the blood system and the molecular mechanisms on pathogenesis of blood diseases, but also provide new ideas for the treatment of clinically relevant

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作者简介: 郭佳妮, 在读硕士研究生, 专业方向: 干细胞与再生医学。E-mail: gjn2205150205@126.com

通讯作者: 王璐, 博士, 研究员, 研究方向: 干细胞与再生医学。E-mail: wanglu1@ihcams.ac.cn

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hematological malignancies.

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自 20 世纪 80 年代美国俄勒冈大学 George Streisinger 首次使用斑马鱼(*Danio rerio*)作为模式生物以来^[1],越来越多的实验室开始使用斑马鱼进行发育生物学及人类疾病的相关研究^[2,3]。斑马鱼是一种淡水硬骨鱼,具有许多独特的生物学优势:体外受精与胚胎透明,便于观察与操作;繁殖能力强且生长迅速,有利于大规模筛选等^[4]。此外,硬骨鱼类和哺乳动物拥有相当数量的同源基因,如 82% 的人类已知致病基因在斑马鱼中存在同源基因^[5],且针对这些基因构建的斑马鱼突变体可作为人类相关疾病研究的动物模型^[6,7]。目前,日渐成熟的基因编辑技术使得斑马鱼疾病模型的构建更加简便快捷。通过 ZFN (zinc finger nucleases)、TALEN (transcription activator-like effector nuclease)以及 CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated 9)技术进行基因编辑,科研人员获取斑马鱼特定基因缺失突变体,进行表型及功能的研究^[8-10]。中国科研人员 Sun 等^[11]应用 CRISPR/Cas9 技术对斑马鱼 1 号染色体基因进行敲除,获得了大量突变体,其中约有 1/4 突变体可以模拟人类相关疾病。基于自身的独特优势以及先进的技术方法,斑马鱼已逐渐成为研究胚胎发育与人类疾病发生机制的重要模型。

1 斑马鱼血液发育过程

血液系统是维持机体生命活动重要的系统之一,为机体提供氧气和营养物质,通过物质交换维持内环境的稳态,同时提供免疫防御与保护。血液系统包含红系细胞、髓系细胞以及淋系细胞等多种成熟血细胞,而这些血细胞均由造血过程产生。造血过程是造血干细胞(hematopoietic stem cells, HSCs)及各类血细胞产生、分化及发育成熟的过程,开始于胚胎发育早期并贯穿整个生命过程^[12]。

脊椎动物造血发育是高度保守的过程,从胚胎发育早期到成体,造血过程的阶段性是一致的,分为初级造血和次级造血两个阶段^[13,14]。初级造血为

早期胚胎发育提供必要的髓系细胞和红细胞。斑马鱼的初级造血发生在两个解剖位置—前侧板中胚层以及由后侧板中胚层发育形成的中间细胞团(intermediate cell mass, ICM),分别生成初级髓系细胞和初级红系细胞^[4]。*scl*、*lmo2*、*flil*、*flkl* 和 *tifly* 等基因对初级造血与血管的发生至关重要^[15-17]; *gata1* 和 *spil*(也被称为 *pu.1*)分别促进红系和髓系的分化^[18,19]。初级造血是一个瞬时过程,很快被次级造血过程替代。次级造血过程始于造血干细胞的产生,位于主动脉-性腺-中肾(aorta-gonad-mesonephros, AGM)区域背主动脉腹侧壁的生血内皮细胞,经过内皮造血转化过程生成 HSCs^[20,21]。随后 HSCs 迁移至尾部造血组织(caudal hematopoietic tissue, CHT)(相当于哺乳动物胎肝),经过短暂扩增之后,大部分 HSCs 迁移并定植于骨髓(相当于哺乳动物骨髓),一部分 HSCs 则迁移到胸腺分化为 T 淋巴前体细胞^[22]。HSC 命运决定的关键基因包括 *runx1*、*scl* 以及 *gata2* 等^[23-26]。与初级造血相似, *gata1* 和 *tifly* 驱动红细胞生成^[15,18], *spil* 和 *c/ebp1* 促进髓系细胞分化^[19,27]。此外, *rag1*、*rag2*、*ikaros*、*lck*、*gata3*、*foxn1*、*irf4a* 和 *rac2* 等关键基因影响淋系细胞的分化^[28-33]。

脊椎动物血液发育是维持机体正常生命活动的动态过程,受多种关键因子和信号通路的精细协同调控,多个造血组织和器官参与其中。在此过程中,任何环节的失调都可能引起血液系统发育或功能缺陷,最终导致血液疾病的发生。因此系统了解并深入探究血液发育的调控机理有助于解析疾病发生机制。

2 斑马鱼血液疾病模型及其应用

血液病是原发于血液系统,或影响造血系统伴发血液异常的一类疾病。血液病种类繁多,且多数为难治性或恶性疾患,近年来发病率有逐渐增高的趋势,其中白血病的发病率和死亡率更是居于我国恶性肿瘤前 10 位。因此,针对疾病临床需求深入开展基础研究,解析疾病发病机制具有重要意义。应

用模式动物, 针对关键致病因子构建疾病模型, 对于人类疾病机制研究、治疗及药物评价至关重要。斑马鱼造血过程及调控机制与哺乳动物高度保守, 结合体外受精、早期胚胎透明、丰富的转基因鱼系等独特优势, 斑马鱼已成为造血发育以及人类疾病研究的常用模式动物之一。在斑马鱼体内模拟致病因子异常变化构建相关血液疾病模型, 可用于肿瘤发生发展可视化研究及高通量化学筛选。本文系统总结了斑马鱼白血病模型和其他多种血液疾病模型的构建, 及其在疾病研究中的应用。

2.1 白血病模型

白血病是一类造血干祖细胞恶性克隆性疾病, 按细胞类型和生长速度主要分为 4 类: 急性淋巴细胞白血病(acute lymphoblastic leukemia, ALL)、慢性淋巴细胞白血病(chronic lymphocytic leukemia, CLL)、急性髓系白血病(acute myeloid leukemia, AML)和慢性髓系白血病(chronic myeloid leukemia, CML)。其中 ALL 可以分为急性 T 淋巴细胞白血病(acute T-lymphocytic leukemia, T-ALL)和急性 B 淋巴细胞白血病(acute B-lymphocytic leukemia, B-ALL)。

2.1.1 T-ALL 模型

T 细胞恶性肿瘤是一类分子异质性疾病, 由复杂的遗传变化所驱动^[34]。淋巴细胞恶性转化的原因主要是异常的染色体易位和异常激活的信号通路^[35,36]。在斑马鱼体内模拟这些改变可以构建 T-ALL 相关转基因斑马鱼系。

原癌基因 *MYC* 影响细胞生长和增殖, 在人类癌症发生过程中发挥重要作用^[37]。第一个 T-ALL 转基因斑马鱼模型是应用斑马鱼 *rag2* 启动子驱动 *EGFP-mMyc* 融合基因表达, 借助该模型美国哈佛大学 Thomas Look 团队首次实时观察 EGFP 标记白血病细胞的发生发展过程^[38]。然而, 该模型的大多数后代在性成熟前便发展为晚期白血病, 无法自然扩繁, 需要通过体外受精进行繁殖, 操作繁琐。为了解决这一问题, Thomas Look 团队构建了条件性转基因鱼系 Tg (*rag2-loxP-dsRED2-loxP-EGFP-mMyc*)。正常状态下该品系不发生白血病, Cre 表达后才可能发生 T-ALL, 然而诱导率仅有 13%^[39]。为了突破低疾病外显率的限制, 该团队引入热休克启动子 *hsp70*

驱动 *Cre* 表达。在受精后 3 天进行热休克处理, 81% 的转基因鱼发生 T 淋巴母细胞性淋巴瘤(T-lymphoblastic lymphoma, T-LBL), 并快速进展为 T-ALL, 该模型大大提高了诱导 T-ALL 的效率^[40]。近期, Jiang 等^[41]应用此疾病模型证明蛋白激酶 Aurora B 能够结合并磷酸化 MYC, 形成 AURKB-MYC 正反馈调控轴, 加快 T-ALL 发生发展。

T-ALL 病例中也常见到 PTEN/PI3K/Akt 通路发生遗传改变^[42]。Thomas Look 团队构建了诱导型 T-ALL 转基因斑马鱼模型, 由 4-羟基他莫昔芬诱导, 激活原癌基因 *MYC* 导致疾病发生。撤除 4-羟基他莫昔芬引起肿瘤消退, 而斑马鱼 *pten* 基因功能缺失突变或持续激活型 *Akt2* 表达则可促进肿瘤继续发展。这些发现说明, 即使 *MYC* 癌基因驱动的信号丢失, Akt 通路激活也足以维持肿瘤生存^[43]。T 细胞前体向 T-ALL 转化的另一个关键基因是 *NOTCH1*, 约 65% 的 T-ALL 患者 *NOTCH1* 基因激活^[44]。应用 *rag2* 启动子驱动 *notch1* 胞内段表达, 成功构建了 T-ALL 模型。同时证明了 Notch 激活与抗凋亡分子 Bcl2 过表达相结合进一步加速了 T-ALL 发生, 提示二者之间的协同作用^[45]。

除了针对已知遗传学改变构建疾病模型, 表型驱动的正向遗传筛选方法也得到广泛应用, 美国犹他大学 Nikolaus Trede 团队应用 N-乙基-N-亚硝基脲(N-ethyl-N-nitrosourea, ENU)化学诱变, 筛选并鉴定了具有遗传倾向的 3 个 T 细胞恶性肿瘤鱼系—*shrek* (*srk*)、*hulk* (*hlk*) 和 *oscar the grouch* (*otg*)^[46]。*srk* 和 *hlk* 突变体作为家族性白血病和淋巴瘤的模型已被多次报道^[47-50]。利用这些突变体进行重叠修饰因子筛选(superimposed modifier screens), 识别影响疾病表型的协同基因。具有低外显率和长潜伏期的杂合突变体适合于筛选肿瘤发生促进因子; 高外显率且早期发病的纯合突变体 *srk* 是筛选肿瘤抑制途径的最佳选择。此外, 这些鱼系的肿瘤临床症状和分子特征与人类 T-ALL 和 T-LBL 相似, 其中胸腺肿瘤症状类似于哺乳动物淋巴瘤模型和人类患者中的纵隔肿块^[46]。从胸腺肿瘤、其他部位的播散性克隆或连续移植后的高度侵袭性肿瘤中分别纯化肿瘤细胞, 比较分析疾病进展各个阶段的细胞特征, 有助于揭示肿瘤转化的分子事件^[46]。这些突变体作为 T 细胞恶性肿瘤的理想模型, 为该疾病的研究提供了新的工具。

2.1.2 B-ALL 模型

染色体易位 $t(12;21)$ 是儿童 B-ALL 中最常见的易位形式,其导致的 *TEL-AML1* (又称 *ETV6-RUNX1*) 融合基因存在于 25% 的患儿中^[51,52]。然而,在小鼠 (*Mus musculus*) 中建立该融合基因 B-ALL 模型并不成功^[53]。后续研究中应用不同的启动子,建立了泛表达或淋系祖细胞特异表达人 *TEL-AML1* 的多种斑马鱼模型,但其中只有少数斑马鱼发生了 B-ALL^[54]。近期研究发现, T-ALL 模型 Tg(*rag2:hMYC*) 会同时发生 B-ALL, 借助淋系特异标记转基因品系 Tg(*lck:EGFP*), 发现 T-ALL 细胞呈现强绿色荧光, 而 B-ALL 细胞则呈现较弱绿色荧光; 此外, *igc1s1* 与 *rag1* 基因分别表达于 B-ALL 与 T-ALL, 可以区分两种细胞簇。基于以上特征, T-ALL 模型 Tg(*rag2:hMYC*) 可用于 B-ALL 疾病的研究^[55]。

2.1.3 AML 模型

染色体重排后产生的致癌融合基因可驱动急性髓系白血病的发生, 如 $inv(8)(p11;q13)$ 导致 *MYST3-NCOA2* 融合基因^[56], $t(8;21)(q(21;22)$ 导致 *AML1-ETO* 融合基因^[57], 以及 $t(7;11)(p15;p15)$ 导致 *NUP98-HOXA9* (*NHA9*) 融合基因^[58]。在斑马鱼中表达常见的致癌融合基因可以构建 AML 疾病模型。

最早的 AML 模型是在斑马鱼胚胎中短暂表达人融合癌基因, 然而这些模型均存在早期致死现象, 无法在成体期进行研究^[59,60]。第一个成功的非胚胎致死 AML 模型是用 *spil* 启动子驱动 *MYST3-NCOA2* 融合基因表达, 该模型中髓系前体细胞广泛侵袭斑马鱼肾脏, 然而 AML 发病率低、潜伏期长^[61]。在时间上控制致癌基因的表达可以有效解决胚胎死亡问题。在斑马鱼胚胎中热激处理诱导 *AML1-ETO* 融合基因表达可模拟人 AML 部分症状, 机制研究表明 *AML1-ETO* 通过 *scl* 影响红-髓系祖细胞分化, 促进粒细胞产生。同时用组蛋白去乙酰酶抑制剂 Trichostatin A 处理可以恢复 *scl* 和 *gata1* 的表达, *AML1-ETO* 引起的粒细胞聚集也得以改善^[62]。应用该模型开展的生物活性化合物筛选, 结果显示环氧合酶-2 (COX-2) 选择性抑制剂—尼美舒利能拮抗 *AML1-ETO* 导致的异常造血分化^[63]。而将热激活驱动与 Cre/loxP 系统结合, 则可以实现特定时间和空间位置诱导癌基因

表达。应用转基因鱼系 Tg (*spil-loxP-EGFP-loxP-NUP98-HOXA9*) 与 Tg (*hsp70:Cre*) 杂交, 可以特异性地在髓系细胞中诱导致癌基因表达。胚胎期过表达 *NHA9* 可干扰早期造血发育, 导致髓系前体占优势; 而在成体期过表达 *NHA9* 可导致 23% 的转基因鱼在 19~23 个月时出现骨髓增殖性肿瘤 (myeloproliferative neoplasms, MPN)^[64]。利用此模型, 加拿大戴尔豪斯大学 Jason Berman 团队发现 DNA 甲基转移酶抑制剂与组蛋白去乙酰化酶抑制剂结合可恢复 *NHA9* 过表达胚胎的正常血液发育^[65], 该发现揭示了 *NHA9* 与表观遗传调控之间的联系, 展示了协同药物组合在 *NHA9* 诱导的髓系疾病中的治疗潜力。

除了染色体重排, 基因表达水平的改变也与 AML 发生密切相关。癌基因 *MYCN* (*N-Myc*) 在 AML 患者体内过度表达, 可作为 AML 不良预后的标志^[66]。Shen 等^[67]构建了由热休克元件 (heat shock elements, HSE) 驱动小鼠 *n-Myc* 基因表达的 AML 模型。*n-Myc* 通过上调 *scl* 与 *lmo2* 增强初级造血, 并通过诱导 *spil* 和 *mpo* 促进髓系细胞扩增, 导致外周骨髓系前体细胞积累。此外, 细胞周期进程改变、糖代谢异常、MAPK/Ras 及 p53 信号途径均参与 *n-Myc* 导致的血细胞恶性转化过程。该模型 AML 发病率高且潜伏期短, 为研究 *MYCN* 致癌作用的分子调控网络建立了有力工具。

2.2 其他血液肿瘤模型

2.2.1 骨髓增殖性肿瘤模型

骨髓增殖性肿瘤 (MPN) 起源于造血干细胞, 表现为骨髓一系或多系血细胞过度增殖, 包括真性红细胞增多症 (polycythemia vera, PV)、原发性血小板增多症 (essential thrombocythemia, ET) 和原发性骨髓纤维化 (primary myelofibrosis, PMF)^[68]。*JAK2*、*CALR*、*RAS*、*CBL* 等基因突变常见于骨髓增殖性肿瘤^[69-71], 其中最高频的突变是 *JAK2* 基因 V617F 的功能获得突变^[72]。

斑马鱼 *jak2a*^{V581F} 突变体可以模拟人 *JAK2*^{V617F} 功能获得突变, 表现为与人类 PV 高度相似的红细胞扩增^[70]。在斑马鱼胚胎表达突变型人 *CALR* 基因会导致胚胎出现类似于 ET 患者的血小板生成增加表型^[73]。然而, 以上两种模型均是在斑马鱼胚胎瞬

时过表达突变基因, 并不能稳定遗传。

在内皮细胞诱导 *RAS* 突变基因表达, 模拟 MPN 表型, 表现为 CHT 区域红系、髓系祖细胞显著扩增, 骨髓中血细胞分化受阻, 以及外周血中红系、髓系祖细胞聚积^[74]。斑马鱼 *irf8* 突变体呈现 MPN 类似表型, 具体表现为髓系前体细胞扩增^[75]。通过正向遗传筛选获得的 *LDD731* 鱼系, 表现为红系、髓系细胞扩增, 且该表型依赖 *Flt3* 信号途径^[76]。这些模型模拟了人类 MPN 表型并初步阐释了其发病机制。

2.2.2 骨髓增生异常综合征模型

骨髓增生异常综合征(myelodysplastic syndromes, MDS)是异质性造血干细胞疾病, 表现为病态造血或无效造血, 以及高风险向 AML 转化^[77]。近年来大量基因组分析显示, MDS 某些亚型与剪接体(spliceosome)或表观遗传因子的突变密切相关^[78-81]。

剪接体突变是 MDS 发病的关键因素, 约 60% 的 MDS 患者中会发生不同形式的剪接体突变^[79]。剪接因子 3B 亚单位 1 (splicing factor 3B subunit 1, SF3B1)是 MDS 中最易突变的剪接体因子之一^[79,81]。斑马鱼 *sf3b1* 功能缺失突变体的初级造血受损, 髓系和红系细胞分化与增殖障碍, 造血干祖细胞产生减少^[82], 该表型与 MDS 患者症状类似^[82]。正向遗传筛选得到的 *cephalophonus(cph)*突变体具有 HSC 产生正常、而髓系和红系细胞发育缺陷的表型, 图位克隆分析鉴定出剪接因子 *prpf8* 是其突变基因^[83]。上述剪接因子突变体具有类似表型, 但也有各自独特的表现, 与临床观察一致, 即不同剪接因子突变的患者有共同的疾病特点, 也有个体化的特征, 斑马鱼模型的使用将有助于个体化精准医疗的实施。

TET2 功能缺失突变常见于髓系恶性肿瘤患者(约 30% 的 MDS 以及约 10% 的再发 AML 病例)^[84]。Gjini 等^[85]研究表明, 纯合 *tet2* 突变体的胚胎期造血正常, 但随着年龄的增长会出现进行性克隆性骨髓增生异常、贫血和髓系祖细胞扩增; 受精后 24 个月, 它们呈现出更严重的 MDS 表型, 如外周血红细胞异常增生。

原癌基因 *c-myb* 是造血细胞增殖和分化的重要调控因子, 其异常表达通常与多种血液疾病相关^[86-88]。转基因斑马鱼 Tg (*c-myb*:GFP)中 *c-myb* 基因过度表达, 髓系细胞显著扩增, 类似于人类 MDS 表型, 且

部分成鱼会发展为 AML 和 ALL。这是由于该模型中过度表达的 *c-myb* 基因影响细胞周期相关基因的表达, 导致造血前体细胞过度增殖。*c-myb* 靶向药物 flavopiridol 可缓解 *c-myb^{hyper}* 胚胎和成鱼的 MDS 样症状。该模型可用于 MDS 发生分子机制的探究以及治疗药物的筛选^[89]。

2.3 斑马鱼血液疾病模型的应用

2.3.1 移植评价实验

应用斑马鱼进行血液肿瘤细胞移植可以用来定义和量化白血病增殖细胞(leukemia-propagating cells, LPCs)以及探究其启动白血病的潜能^[90]。斑马鱼的移植评价实验具有诸多优势: 使用受精后 4 周内的斑马鱼胚胎进行移植不需要免疫抑制, 因为该时期斑马鱼缺乏成熟的适应性免疫系统^[91]; 透明的胚胎或成体鱼 *Casper* 与多种荧光转基因品系相结合, 促进了活体成像技术在肿瘤发生发展研究中的应用^[92]。

首次移植实验是应用 T-ALL 模型 Tg (*rag2-EGFP-mMyc*), 将 EGFP 标记的白血病细胞移植到辐射后野生型成年斑马鱼腹腔内, 这些细胞在腹腔注射后 14 天开始扩散, 14~26 天归巢于胸腺^[38]。连续移植后诱发疾病的能力是肿瘤细胞自我更新的标志。T-ALL 细胞连续移植揭示了大部分 T-ALL 细胞具有启动白血病的潜能。此外, 大规模的单细胞移植实验则证实了这一启动潜能呈现差异性, 即 0.1%~15.9% 甚至更少的白血病细胞有能力重建肿瘤^[90]。

复发 T-ALL 患者中经常发生克隆进化, 导致肿瘤更具侵袭性, 这一现象与 LPCs 的遗传多样性及其增强的白血病启动潜能相关^[93,94]。研究表明, Notch 信号能促进 T-ALL 癌前 T 细胞克隆扩增, 提高积累必要突变并完全转化为 LPCs 的可能性和速度^[95]。大规模细胞移植筛选实验显示单个克隆之间存在功能变异, 少数克隆随着时间的推移提高了生长速度和扩增潜力。Blackburn 等^[96]研究证明, 克隆进化激活 Akt 信号通路促进 T-ALL 的生长, 同时可促使肿瘤细胞对地塞米松产生耐药性。以上研究提示, 克隆进化促使 T-ALL 对化疗产生耐药性, 而且这可能发生于药物暴露之前。

斑马鱼移植受体的免疫系统可被地塞米松或 γ 射线短暂抑制, 却无法进行长期移植; 且仅可用于

同源移植,限制了移植模型的广泛应用^[97]。为了解决这些问题,美国哈佛大学 David Langenau 团队构建了免疫缺陷斑马鱼模型—*rag2*^{E450fs} 突变体。该突变体中功能性 T 细胞和 B 细胞数量减少,但仍能存活和繁殖,并且可进行多种组织与癌细胞长期稳定移植。但是该模型纯合子斑马鱼不能繁殖,以及个体间 B 细胞缺陷差异极大,可能影响植入潜能^[98]。免疫缺陷斑马鱼模型 *jak3*^{P369fs} 突变体和 *prkdc*^{D3612fs} 突变体,分别导致 T 细胞与 NK 细胞、成熟 T 细胞和 B 细胞的缺失,两种突变体均具有植入能力,但只有 *prkdc* 纯合突变体可以繁殖,并且在细胞移植后存活^[97]。斑马鱼 *prkdc*^{-/-}, *il2rg*^{-/-} 双突变体中 T 细胞、B 细胞和 NK 细胞缺陷,David Langenau 团队应用该免疫缺陷突变体建立并评估了肿瘤移植模型,能够重现多种病人来源的肿瘤生长迁移等情况,并可在单细胞水平进行活体实时研究^[99]。

2.3.2 化学药物筛选

斑马鱼模型是进行高通量药物筛选的理想选择,主要基于两个优点:(1)整体动物模型,可以针对特定生物学事件发现活性化合物和药物靶标;(2)全面评估化合物的活性和副作用,排除具有明显毒副作用的化合物,缩短药物研发周期^[100-102]。

研究表明前列腺素 E2 (prostaglandin E2, PGE2) 的代谢活性衍生物二甲基前列腺素 E2 (16,16-dimethyl-PGE2, dmPGE2) 可以增加 HSCs 数量,同时也可促进辐射损伤成体鱼中骨髓的恢复^[103]。此外, dmPGE2 可提升小鼠骨髓 HSCs 移植重建能力^[103]。临床前分析显示, dmPGE2 能显著促进体外人造血细胞集落形成,提高异种移植后人脐血干细胞 (human cord blood, hCB) 的植入效率^[104]。人类脐带血移植的临床研究显示,经 dmPGE2 处理的脐带血细胞具有持久、多系重建潜能,且安全性高,移植后患者中性粒细胞的恢复速度也大大加快^[105]。美国犹他大学 Nikolaus Trede 团队应用淋系转基因斑马鱼进行药物筛选,确定小分子化合物 Lenalidekar (LDK) 可以有效消除不成熟的 T 细胞而不影响正常细胞的细胞周期,且可延长大部分 T-ALL 成鱼的生存期^[106]。在小鼠模型中, LDK 也表现出了减缓疾病进展的功效。同时对于直接取自临床原发性白血病 (包括难治性 B-ALL 和慢性粒细胞白血病) 患者的样

本, LDK 可以杀死其中大部分的白血病细胞^[106]。这项工作证明了使用斑马鱼筛选抗肿瘤药物的实用性,同时 LDK 的发现也为白血病靶向治疗提供了新方向^[106]。

接受异种移植的斑马鱼也是药物筛选的一个重要模型。将人类白血病细胞移植到斑马鱼胚胎中用于筛选非致畸的白血病治疗药物,结果显示伊马替尼和奥沙福林可消除白血病细胞,且对受体胚胎无毒性;而全反式维甲酸和 4EGI-1 表现出致畸作用,不能作为抗白血病药物^[107]。

3 结语与展望

斑马鱼血液疾病模型的应用为人们了解疾病的病理生理学、基因型与表型的相关性以及探索治疗方案提供了新的途径。应用正反向遗传学方法获得的斑马鱼血液疾病模型,可用于化学筛选靶向治疗药物,或进行异体移植研究白血病增殖细胞的功能等(图 1)。然而斑马鱼模型的应用通常缺乏对肿瘤发病的时空控制,这限制了其在肿瘤进展和转移研究中的应用。最近一项研究开发了一种成年斑马鱼转基因电穿孔(transgene electroporation in adult zebrafish, TEAZ)技术,可以在成鱼体内定时定点导入特定的 DNA,如原癌基因、CRISPR/Cas9 基因编辑组件以及特定启动子驱动荧光蛋白的 DNA 载体^[108],可以实现特定组织和发育时期引入遗传改变。斑马鱼模型应用的另一个限制是缺乏可靠的细胞表面标记及抗体,目前大多数造血细胞分选都是基于转基因标记,极大地限制了特定谱系亚群的分选^[109]。此外,人们对斑马鱼造血微环境的了解仍然很少,造血细胞之间的相互作用、与微环境细胞的相互作用及其与血液疾病的关系值得进一步探究。

异种移植模型极大地加深了人们对白血病发生和干细胞生物学的理解。患者来源的异种移植植物保持了人类癌症固有的克隆异质性,提供了优于体外系统的微环境,这对药物的开发与临床转化有重要意义^[110,111]。虽然斑马鱼异种移植平台已初步建成^[99,112,113],但是人类造血干祖细胞在斑马鱼体内仅能短暂地存活^[114]。最近,一种人源化斑马鱼模型可以表达多种人类造血特异性的细胞因子,从而促进了受体中造血干祖细胞的存活、自我更新和多向分

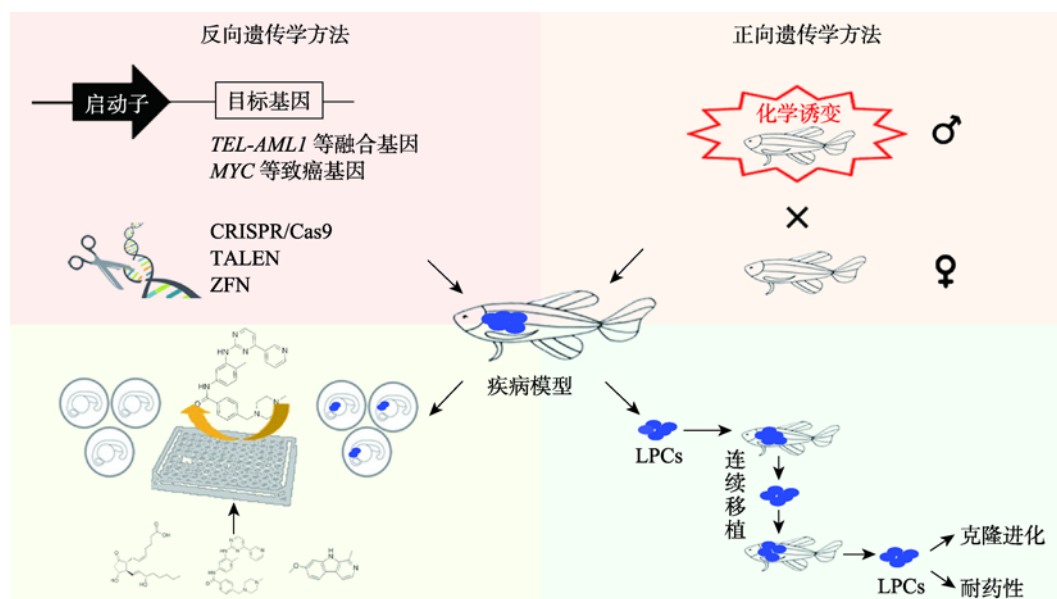


图 1 斑马鱼血液疾病模型构建与应用

Fig. 1 Generation and application of zebrafish blood disease models

应用正反向遗传学方法构建斑马鱼血液疾病模型, 可用于化学药物筛选或肿瘤细胞移植评价研究。

化, 且移植白血病细胞表现出向造血组织的归巢, 更准确地模拟了人类白血病的行为^[115]。除了血液系统肿瘤, 在其他人源肿瘤的移植方面斑马鱼也显示出独特的优势: 活体成像可以对肿瘤生长、转移、血管生成以及肿瘤起始细胞进行动态分析, 斑马鱼也为高通量筛选抗癌药物提供了一个经济有效的平台^[99,116,117]。

疾病模型的应用有助于探索个体化精准医疗。最近, 研究人员利用斑马鱼拯救了一位患有淋巴管疾病的 12 岁男孩的生命。全外显子测序发现该患儿 X 染色体上 *ARAF* 基因发生错义突变, 过表达携带这种突变的人源基因导致斑马鱼淋巴管的过度生长, 化学筛选证明 *MEK* 抑制剂对该表型有回救作用; 这不仅明确了 *ARAF* 基因突变导致该患儿发生疾病, 也寻找到了有效的治疗药物^[118]。这是通过斑马鱼实现精准医疗的成功案例, 但是由于生命活动的关联性与复杂性, 基因的改变往往会造成级联反应, 如何将斑马鱼的研究成果过渡到人类医疗中仍然需要持续的探索。

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