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鱼类早期红细胞发育与抗逆性状形成的研究进展

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摘要: 随着水产领域的设施化和高密度集约化养殖的发展, 培育具有适应集约化养殖模式的耐低氧(抗非生物逆境)、抗病(抗生物逆境)、低应激等性状的鱼类新品种是解决水产养殖产业问题的关键。造血系统是维持鱼类正常生命活动最重要的系统之一, 其中, 红细胞参与鱼类氧气、营养物质运输和免疫防御应答等功能, 在鱼类抗病和耐低氧过程中发挥重要调控作用。红细胞发育及其功能调控的分子元件是鱼类抗病抗逆性状形成的重要遗传基础。文章以鱼类红细胞发育为切入点, 关注调控红细胞发育、成熟、功能的经典信号和分子, 并从氧气运输和免疫防御应答两方面详细阐述了鱼类红细胞的生理功能, 同时, 关注鱼类在逆境胁迫下的红细胞发育及其功能调控的适应性机制。文章探讨了鱼类红细胞发育及其抗逆性状形成的遗传基础研究, 鱼类逆境胁迫下的红细胞生成及其生理响应, 为水产养殖动物抗逆新种质创制提供理论依据。

关键词: 造血系统; 红细胞发育; 红细胞免疫; 耐低氧; 抗病; 环境胁迫; 抗逆育种

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鱼类在全球不同国家的水产品消费中占比均超六成, 中国是世界最大的水产品生产国、消费国、进口国(按贸易量计)和出口国^[1]。2022年中国水产品总产量6865.91万吨, 其中养殖产量同比增长2.62%, 捕捞产量同比增长3.17%^[2]。水产品产量增速主要依赖于养殖面积的增加和单位面积产量的增加。近年来, 随着工业用地、住宅开发需求强烈^[3]和环境资源保护意识的提升, 水产品养殖面积在部分省份呈现明显下降趋势。为了促进水产品产量的持续增长, 渔业养殖进一步集约化、生态化、现代化。

鱼类集约化养殖具高密度和疾病传播快等特征, 易于产生新型环境胁迫(逆境), 如营养元素矿物质富集、低氧、高密度(非生物逆境), 以及病原微生物富集(生物逆境)等, 严重制约了养殖鱼类的产量增长^[4]。随着水产集约化养殖的快速发展, 如何

利用鱼类抗逆优良性状的遗传基础及鱼类在逆境中产生的适应性遗传和表观遗传变异(逆境适应遗传变异)而创制和筛选抗逆等优良种质以适应新型渔业环境胁迫, 是水产养殖领域面临的挑战。

鱼类红细胞广泛参与机体营养、氧气输送、细菌吞噬免疫、感染防疫等内环境的稳态维持。终生拥有细胞核是鱼类红细胞最典型的特征。尽管细胞核的存在使得鸟类、两栖类、鱼类红细胞的氧气运输能力有所损失^[5], 但细胞核和各种细胞器中的转录调控和代谢反应等都使得具有有核红细胞的生物具有更强大的应激调节能力^[6]。鱼类在集约化养殖中, 高密度的养殖导致溶氧量较低和病原微生物富集等环境胁迫问题, 进一步导致鱼类缺氧及病害发生, 制约了养殖鱼类的产量增长, 严重时可能导致重大经济损失^[7]。为此, 本文聚焦鱼类红细胞发育, 重点关注鱼类红细胞在缺氧及病原微

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生物胁迫时的适应调节机制, 关注调控鱼类红细胞发育及其功能的分子元件在鱼类抗逆性状形成中的调控功能, 为水产养殖鱼类抗逆新种质创制提供理论基础。

1 鱼类红细胞发育

红细胞是鱼类最丰富的血细胞类型^[8], 广泛参与系统营养转运、气体运输、感染防疫等重要生理过程。2000年前后, 基于造血干细胞(Hematopoietic stem cells, HSCs)的基本属性对HSC下游祖细胞群体进行分类形成了最经典的造血分化树模型^[9], 结合红细胞发育及其成熟过程, 硬骨鱼类的红系造血谱系分化的主要阶段为造血干细胞(HSC)、巨核-红系祖细胞(Megakaryocyte erythroid progenitors, MEP)、红细胞暴发形成单位(Burst forming unit-erythroid, BFU-E)、红细胞集落形成单位(Colony forming unit-erythroid, CFU-E)、原红细胞、成红细胞等^[10]。伴随单细胞技术及更精细的细胞表面分子标记的发展与应用, HSC造血池(HSC pool)的概念取代了同质化的HSC, 其包含不同移植属性的HSC和由HSC直接产生的具有髓系、淋系多种分化潜能的多能祖细胞(Multipotent progenitor cell, MPP), 强调其作为一个集体的储备和平衡状态^[11, 12]。

鱼类造血发育过程主要可分为初级造血(Primitive wave)和次级造血(Definitive wave)两个阶段。在模式动物斑马鱼中的研究发现, 初级造血起源于腹侧中胚层, 包括前侧板中胚层(Anterolateral mesoderm, ALM)和后侧板中胚层(Posterolateral mesoderm, PLM)两部分。前者产生初级髓系细胞^[13], 后者在约18hpf (Hours post fertilization)时发育形成类似于哺乳动物卵黄囊的中间细胞团(Inner cell mass, ICM)^[9]。ICM区域主要形成初始红系细胞和部分髓系细胞, 为早期胚胎提供免疫保护及携氧转运功能细胞^[14]。初级造血具有非HSC依赖性的特征, 通过直接形成初级红系和髓系细胞维持早期发育的稳态调节^[11]。这种短暂性的造血过程随后会被HSC依赖的次级造血所取代。次级造血所产生的造血干前体细胞(Hematopoietic stem and progenitor cells, HSPCs)在胚胎及成体中分化产生所有谱系血细胞^[15](图1)。

在次级造血阶段, 造血干细胞起源于背主动脉(Dorsal aorta, DA)的腹壁, 即主动脉-性腺-中肾区(Aorta-gonad-mesonephros, AGM)^[16]。自24hpf起, AGM区部分内皮细胞特化形成生血内皮细胞(Hemogenic endothelial cells, HECs), 经内皮-造血转换(Endothelial-to-hematopoietic transition, EHT)发生

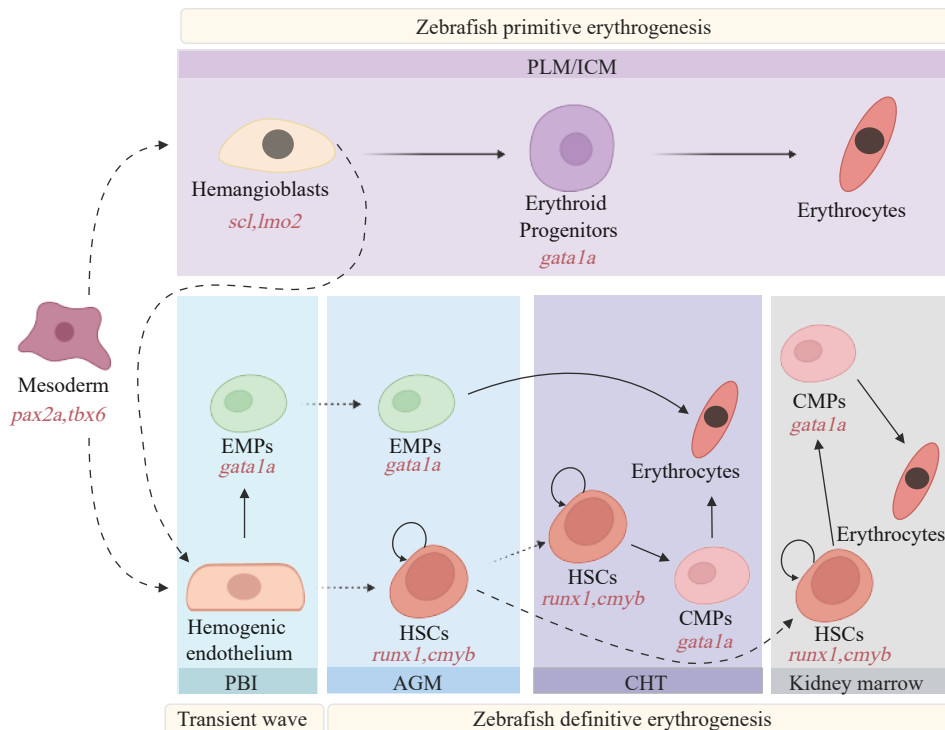


图1 鱼类发育过程中的红细胞生成

Fig. 1 Erythropoiesis during fish embryogenesis

红系祖细胞erythro-myeloid progenitors (EMPs); 造血干细胞 hematopoietic stem cells (HSCs); 共同髓系祖细胞common myeloid progenitors (CMPs); 后外侧中胚层the posterior lateral mesoderm (PLM); 中间细胞团intermediate cell mass (ICM); 后血岛posterior blood island (PBI); 主动脉-性腺-中肾aorta-gonad-mesonephros (AGM); 尾部造血组织caudal hematopoietic tissue (CHT)

细胞形态变化^[17], 形成单个圆形的造血干前体细胞(HSPCs), 后迁移至鱼类尾部定植形成尾部造血组织(Caudal hematopoietic tissue, CHT)^[18], 部分细胞会迁移到胸腺分化成T淋巴细胞。HSPCs在CHT中进行短暂扩增后, 从3 dpf (Days post fertilization)开始, 移动至肾脏, 建立HSPCs库并维护斑马鱼的终生造血功能^[9](图1)。斑马鱼红细胞荧光标记模型常被用来研究红细胞发育过程。其中, 应用最广泛的是*Tg (gata1: DsRed)*^[19], 其通过*gata1* (*GATA binding protein 1*)启动子来驱动红色荧光蛋白DsRed的表达, *Tg (drl: EGFP)*和*Tg (lcr2: EGFP)*^[20]等荧光品系也可见红细胞被特异性标记绿色荧光。

2 鱼类红细胞生成的调控机制

2.1 重要转录调控因子

鱼类造血系统发育是由各种转录因子与信号通路共同作用来调节的, 其动态过程精密而复杂。在初级造血中, *scl* (*T-cell acute lymphocytic leukemia 1*)和*lmo2* (*LIM domain only 2*)基因是调控初级造血与血管发生的关键因子^[21]。在*scl*基因敲除的斑马鱼胚胎中, 背主动脉血管标记基因的表达量减少, 肌节间血管不能形成, 初级造血和次级造血缺失^[22]。而*lmo2*基因的敲除会导致PLM造血功能的完全丧失, 包括初始红系细胞生成^[23]。此外, 定位于血管内皮细胞中的*fli1* (*Fli-1 proto-oncogene*)可以诱导*scl*、*lmo2*的表达^[24]。鱼类内皮基因*flkl* (*kinase insert domain receptor like 1*)及其造血干前体细胞的发生和功能重要转录因子*cmyb* (*v-myb avian myeloblastosis viral oncogene homolog*)和*runx1* (*RUNX family transcription factor 1*)决定主动脉生血内皮的特征^[25]。模式鱼类研究发现, 鱼类胚胎在24—48 hpf时*cmyb*与*runx1*在AGM区共表达, 标记造血干前体细胞^[26]。*cmyb*在次级造血中发挥重要作用, 而*runx1*则是AGM中HSPCs形成所必需的转录因子^[27]。在造血过程中, *gata1*和*pu.1* (*Spi-1 proto-oncogene b*)分别调控造血干前体细胞向红系和粒系分化, 并相互拮抗^[28], *Lmo2*介导*Scl*和*Gata1*结合形成转录激活复合体, 共同促进血红蛋白基因转录^[29]。

2.2 信号通路

除了经典的转录调控因子, Wnt/ β -catenin和BMP信号通路等在鱼类红细胞的发育分化过程中也发挥重要调控作用。

Wnt/ β -catenin通路 Wnt/ β -catenin通路调控很多细胞生物学过程, 包括激活基因表达、调控生长和细胞迁移等^[30]。Wnt通路最早在无翅果蝇突变体被发现与出翅转变相关^[31], 其后被发现通过控

制胚胎干祖细胞的自我更新和命运决定来决定胚胎发育过程和图式形成^[32]。

Wnt是一类分泌性信号蛋白, 通过结合Frizzled胞外结构域使LRP磷酸化而完成Wnt通路细胞内外信号传导。在无Wnt信号时, Axin/GSK-3/APC复合物促进细胞内 β -Catenin蛋白磷酸化后泛素化, 而被蛋白酶体降解, 核内转录因子TCF与Groucho充当Wnt靶基因的转录共阻遏物。而Wnt信号存在时, 磷酸化的LRP激活Dvl抑制 β -Catenin蛋白降解, 进而 β -Catenin入核, 取代Groucho结合TCF, 解除Wnt靶基因的转录抑制。研究发现鱼类HSPC的出现依赖于Wnt/ β -catenin信号通路^[33]。Wnt拮抗剂Dkk1过度表达会导致36hpf时*cmyb*阳性HSPCs减少, 而Wnt8的过度表达会有效促进HSPCs的扩增^[34]。Axin是复合体的核心成分, 使用小分子使Axin和LRP6紧密连接以刺激Wnt通路后可观察到*cmyb*阳性HSPCs数量增加^[35]。 β -Catenin结合域缺失的TCF转录因子(*dntcf*)表达亦导致细胞无法响应Wnt信号从而表现出HSPCs数量减少^[36]。在VEC-Cre小鼠胚胎内皮细胞中, β -Catenin缺失会影响AGM区的造血内皮转换过程, 另外, *Wnt3*突变严重减少了该过程HSPCs的产生数量^[37]。

Wnt通路对红细胞生成调控的研究更多集中于其改变红细胞命运决定的关键转录因子的表达, 多在HSPC转向红系谱系的过程中发挥调控作用。研究发现Wnt信号可以下调HSPC中编码PU.1的*Sfp1*, 进而增强HSPC的红系谱系分化^[38]。源自卵黄囊的原始红系祖细胞(EryP-CFC)可检出Wnt通路的多种信号分子^[39], 小鼠胚胎干细胞(Embryonic stem cells, ESCs)体外培养实验中, β -Catenin激活后红系谱系标记物GATA1上调, 进而刺激原始红细胞谱系的生成^[40]。WNT/ β -Catenin信号传导中的转录因子TCF4可结合斑马鱼基因*scl*和*lmo2*的启动子并调节其表达^[41]。在非洲爪蟾中也报道了Wnt信号对胚胎红细胞生成的调控, *Wnt4*的缺失可能会导致Wnt/ β -Catenin和非经典Wnt信号的功能下调进而阻遏红细胞生成^[42]。这些研究显示, 经典Wnt信号正向调控红细胞发生。研究还发现, Wnt信号在造血发生过程中以剂量依赖性方式参与HSPCs谱系分化^[43]。

BMP通路 骨形态发生蛋白(BMP)是TGF- β 家族的成员, 在AGM区HSPCs的诱导和维持过程中发挥着关键作用。BMP配体与其受体结合使R-Smad (鱼类中的Smad1、Smad5和Smad9)被磷酸化, 激活的R-Smad与作为Co-Smad的Smad4结合并入核, 通过与BMP响应元件(BMP responsive elements, BRE)结合来激活靶基因的转录。BMP是个

体腹侧中胚层发育的必需蛋白,因此BMP通路被认为通过腹侧中胚层发育直接影响鱼类初级造血。斑马鱼BMP通路的**bmp2** (*swirl*)和**bmp7** (*snailhouse*)纯合突变体无法发育出成熟红细胞,包括成熟红细胞^[44],**somitabun**^{dtc24} (*sbn*)^[45]和**captain hook** (*m169*)^[46]等Smad缺陷相关突变体中均可观察到不同程度的腹侧组织发育缺陷。小鼠**Bmp4**突变导致的致死胚胎中可以观测到初级造血组织的明显减少^[47], ESCs的体外实验则表明BMP4可以调节其体外造血发育过程^[48]。本世纪初,研究人员在非洲爪蟾中发现,BMP信号传导下游靶基因包含对红系发育至关重要的转录因子,如Gata1、Gata2、Scl、Lmo2等^[49],说明BMP通路通过R-Smad介导,直接或间接调控红系发育关键基因,进而调控红细胞生成,并存在发育阶段或信号强度的差异性特征^[50]。

2.3 表观修饰

表观遗传学被定义为不改变DNA核苷酸序列的基因表达调控机制的研究^[51],因其参与调控生物学过程的广泛性和灵活性,近年来是解析分子机制的重点研究领域。个体的早期发育依赖复杂的调控机制及其调控网络,这个过程由基于中心法则的转录调控因子与基于结构修饰的表观调控共同参与,以促进或抑制基因表达。

细胞染色质由DNA和组蛋白组成。许多共激活因子或共抑制因子催化组蛋白尾部翻译后修饰(如磷酸化、甲基化、乙酰化等)的发生与去除或核小体重塑,从而实现靶基因表达的调控。NuRD (Nucleosome remodeling and deacetylase)复合体是一个多功能的表观遗传学调节复合体,它结合了染色质重塑和组蛋白去乙酰化功能,乙酰化修饰通常被认为可松弛染色质结构,从而促进基因转录。组蛋白去乙酰化酶(Histone deacetylase, HDAC)承担NuRD复合体的去乙酰化活性,可通过去除组蛋白H3和H4赖氨酸残基上的乙酰基来抑制转录。HDAC抑制剂(VPA)可以抑制NuRD活性,在非洲爪蟾原肠胚初期处理会阻止初级红细胞生成并导致异常的血管生成^[52],而NuRD成分的过度表达会增强斑马鱼胚胎中**scl**和**lmo2**的表达^[53]。组蛋白甲基化修饰相关的酶主要分为添加修饰的组蛋白甲基转移酶(Histone methyltransferases, HMTs)和移除修饰的组蛋白去甲基化酶(Histone demethylases)两种。LSD (Lysine demethylase)蛋白家族是重要的组蛋白去甲基化酶,TAL1 (SCL)将LSD1复合物和HDM招募到其目标启动子或激活子上,LSD1复合物发挥去甲基化活性去除组蛋白H3K4me2的甲基化修饰去除,而下调LSD1可能导致H3K4me2的增加而H3K4me1

的减少进而抑制了TAL1下游目标基因的表达,从而抑制红系分化程序^[54]。此外,LSD1也被发现可与GATA2和TAL1形成复合物介导组蛋白H3K4me1的去甲基化,以抑制未分化细胞中GATA1的表达^[55]。斑马鱼中一项大规模的反向遗传筛选研究发现了15个调节初始红系祖细胞发育的因子和29个调节终末红系祖细胞发育的因子,这些染色质因子与SWI/SNF和ISWI染色质重塑、SET1甲基转移酶、CBP-p300-HBO1-NuA4乙酰转移酶、HDAC-NuRD脱乙酰酶和Polycomb抑制复合物相关^[56]。

这些组蛋白表观修饰相关酶的作用并非完全依赖其表观修饰或去修饰活性,其也可作为普通的转录因子参与造血调控。如EZH2 (Enhancer of zeste homolog 2)是多梳抑制复合物2 (Polycomb repressive complex 2, PRC2)的一个亚基,其通过SET结构域调控H3K27me3修饰,进而完成基因沉默转录调控^[57]。小鼠肝脏中**ezh2**特异性敲除模型中可观察到红系祖细胞减少^[58],而斑马鱼**ezh2**纯合突变体呈现出初级和次级造血受损表型,EZH2被发现作为转录因子直接结合启动子上E-box调节**lck** (*lymphocyte cell-specific protein-tyrosine kinase*)进而促进正常造血^[59]。

除较为灵活的组蛋白修饰外,DNA甲基化修饰变化在造血细胞命运决定和谱系定向中也发挥重要调控作用。DNA甲基转移酶DNMT3a和DNMT3b负责CpG岛上的从头甲基化,而DNMT1在DNA复制后保留DNA甲基化模式^[60]。DNMT3A是小鼠造血细胞中主要的从头DNA甲基转移酶,并且DNMT3B和DNMT3L主要充当伴侣以增强DNMT3A的活性^[61,62]。此外,也有染色质重塑等表观遗传途径调控红细胞发育,如SMARCA1作为ATP依赖性染色质重塑因子,其缺失会导致**scl**、**gata1**和**hbbe3** (*hemoglobin beta embryonic-3*)表达明显下调^[56]。

3 鱼类红细胞对胁迫的生理响应

3.1 低氧胁迫与携氧运输

鱼类有氧代谢依赖水中溶氧(Dissolved oxygen, DO),故DO是水产养殖中的主要环境限制因素和检测指标^[63]。幼鱼的缺氧适应与氧运输、氧化还原过程、血红蛋白生物合成过程、红细胞发育和细胞铁离子稳态等生物过程密切相关^[64]。在集约化养殖中,由于鱼类密度大,水体中DO消耗快,加之水体循环不畅可能导致藻类大量繁殖,在夜间或气候条件不佳时,鱼类容易发生缺氧情况^[65]。大多数的鱼类具有一定的低氧适应能力,但严重缺氧的持续存在将导致其大量死亡,因此鱼类通过增加红细胞

携氧能力和增加红细胞数量, 进而应对低氧胁迫环境。

低氧胁迫下鱼类红细胞携氧能力调节

硬骨鱼类拥有五种不同的珠蛋白类型, 其中血红蛋白在红细胞中承担氧气运输功能^[66]。与从空气中获取氧气的脊椎动物相比, 鱼类通常要应对更复杂的环境氧含量变化, 其血氧运输的调节由多种功能不同的血红蛋白(Hemoglobin, Hb)亚型的协调表达来介导^[67]。脊椎动物缺氧应激可通过调节Hb氧亲和力提高红细胞携氧能力, 其变化与翻译后水平的Hb构型异质性、细胞内变构调节剂等因素相关。在金鱼(*Carassius auratus*)^[66]、帆鳍莫莉(*Poecilia latipinna*)^[68]、大菱鲆(*Scophamus maximus*)、鲷(*Sparus aurata*)^[69]等物种中观察到的低氧胁迫下Hb氧亲和力及其表达水平调节证明其响应低氧胁迫具有鲜明的物种差异。同时, 大多数硬骨鱼在成熟红细胞中可表达多种结构不同的Hb同工型, 其开关调控也与低氧耐受相关^[70]。此外, 鱼类在生境中自然具有的高亲和力血红蛋白亚型多为演化塑就, 这种现象更多在长期受到缺氧环境选择的物种或群体中被观察到, 如高原裂腹鱼^[71]、维多利亚湖慈鲷(*Haplochromis ishmaili*)^[72]、海洋鱼类^[73]等。

低氧胁迫下鱼类红细胞分化调节

为了应对复杂多变的氧气供应环境, 后生动物演化出高效的气体信号传导系统用以感受胞内氧含量变化, 如mTOR、ERK、NF- κ B等^[74]。在细胞水平上, 低氧诱导因子(Hypoxia-inducible factor, HIF)作为重要的转录调控因子参与多种信号通路, 是氧稳态时空调节中的特异性和主导性调控因子, 其通路的基因靶标在细胞、个体的局部和全身响应缺氧中均发挥关键调控作用^[75]。HIF蛋白具有异二聚体特征, 由氧气调节相关的HIF- α 亚基和组成型表达的HIF- β 亚基组成, 前者活性对氧气变化敏感, 而后的蛋

白质水平稳定, 对氧气变化的响应不明显^[76]。在正常氧气条件下, HIF- α 由脯氨酰羟化酶(Prolyl hydroxylases, PHD)和(Factor inhibiting hif, FIH)羟基化后结合pVHL (Von Hippel-lindau), 招募E3泛素连接酶复合体(VHL, Elongin B, Elongin C, VBC)而介导蛋白酶体降解^[77]。而在缺氧条件下, PHD活性受到抑制, HIF- α 蛋白积累与HIF- β 形成二聚体, 进入细胞核作为转录因子发挥作用与缺氧响应元件(Hypoxiaresponsive element, HRE)结合进而调控下游缺氧响应基因的表达^[78](图2)。在水生动物中, 已鉴定出HIF通路关键成员的同源物, 包括HIF、PHD、FIH、VHL等^[79]。

低氧诱导因子HIF

目前对缺氧条件下HIF-1的研究最为广泛, 鳙^[80]、罗非鱼^[81]、鲈^[82]、团头鲂^[83]等多种硬骨鱼的\alpha基因全长序列已被克隆或研究。早期研究认为低氧暴露会增加\alpha水平, 但后续多项研究^[78, 84, 85]表明在不同时期胚胎中其激活的阈值存在差异, 在缺氧条件下的表达差异可能与低氧胁迫开始时鱼类的发育时期有关^[86]。在造血内皮转换过程中, HIF-1 α 通过激活Notch1信号传导而上调gata2b, 正向调节EHT过程^[87], 其也被发现可以直接与原始红细胞中gata1的3'侧翼区的HRE结合而激活gata1转录^[88]。

脯氨酰羟化酶PHD

PHD2具有MYND型锌指结构域及保守半胱氨酸和组氨酸^[89], 作为大多数鱼类细胞中最丰富的PHD亚型, 近年来其在多种经济鱼类(如鳊^[90]、鲢^[89]、团头鲂^[91]等)的低氧应激反应中被广泛关注。不同亚型PHD对哺乳动物中的HIF- α 表现出不同的羟基化偏好^[92], 其在不同氧气浓度下的贡献度亦可能存在差异^[90]。

HIF抑制因子FIH

HIF通路内部存在一种应对低氧胁迫下HIF激增的反馈调控机制(图3)。FIH-1是一种Fe²⁺和2-酮戊二酸(2-oxoglutarate, 2-

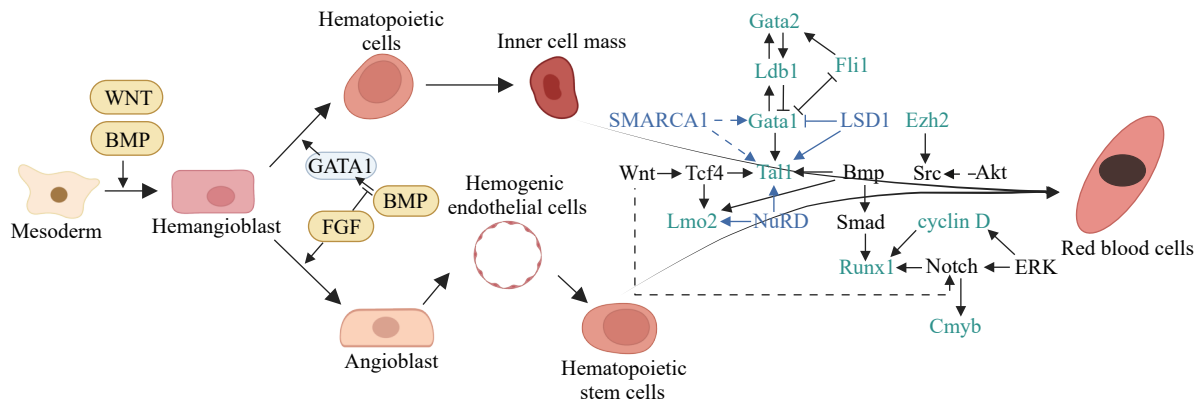


图2 鱼类早期红细胞生成的调控机制

Fig. 2 Regulating mechanisms of embryonic erythropoiesis

OG)依赖性双加氧酶,在HIF通路中发挥类似PHD的羟基化功能,区别在于其对HIF- α 的羟化并不导向蛋白酶体降解,而是通过抑制转录共激活因子P300/CBP的募集以抑制HIF- α 活性^[93]。根据在尼罗罗非鱼^[81]和斑点叉尾鲷^[94]中观察到的现象可以

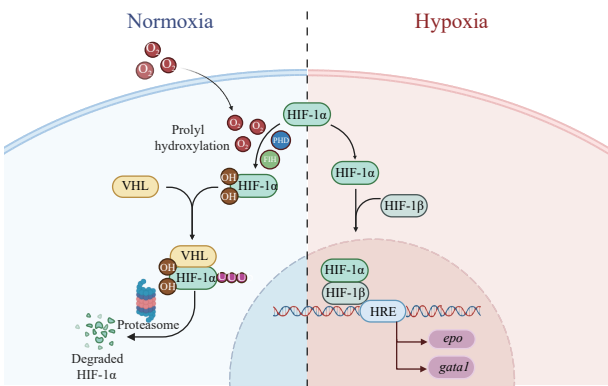


图3 鱼类HIF低氧应激通路调控低氧条件下的红细胞生成
Fig. 3 Hypoxia-inducible factor (HIF) pathway in regulating erythropoiesis in fish under hypoxia

推测,长时间缺氧时可能存在一种对HIF蛋白转录活性进行微调的机制,既防止HIF降解导致的浪费,又可以上调FIH避免HIF通路过度激活而导致的造血异常^[95]。

促红细胞生成素EPO 鱼类中HIF下游与红细胞生成相关重要的靶基因之一是促红细胞生成素基因(Erythropoietin, *epo*)。在硬骨鱼中,EPO最早在红鳍东方鲀(*Takifugu rubripes*)中被鉴定,是红系分化和最终成熟的必要条件^[96]。*epo*的表达具有发育阶段特异性、细胞类型特异性和缺氧诱导性特征^[97],仅参与晚期CFU-E到红细胞的发育过程^[98],而对造血干细胞向红系谱系定型并不重要^[99],目前已有一些文献关注到鱼类初级造血阶段红细胞的产生受到外周细胞和组织分泌的EPO的调节^[100,101]。缺氧信号通过HIF应激通路激活*epo*表达,EPO释放到血浆后结合细胞表面受体EPOR并使之二聚化,同时激活JAK2-STAT5、PI3K-Akt和MAPK-ERK通路^[102],介导GATA-1磷酸化并增强其转录活性^[103],从而加速红细胞的发育、分化和成熟。同时,EPO

表1 部分鱼类红细胞上模式识别受体应激调控

Tab. 1 Stress regulation of pattern recognition receptors in erythrocytes in fish				
模式识别受体 PRR		抗原 Antigen	品种 Species	来源 Reference
Toll样受体(TLR)	<i>tlr2</i>	嗜水气单胞菌	南亚野鲮 (<i>Labeo rohita</i>)	[106]
	<i>tlr3</i>	嗜水气单胞菌	南亚野鲮	[106]
		聚胞苷酸	尼罗罗非鱼 (<i>Oreochromis niloticus</i>)	[107]
		鳃弧菌	日本牙鲆 (<i>Paralichthys olivaceus</i>)	[108]
		鳃弧菌	欧洲鳗鲡 (<i>Anguilla Anguilla</i>)	[109]
		传染性胰腺坏死病毒, 重组VHSV 糖蛋白G片段16, 聚胞苷酸 鱼正呼肠孤病毒	虹鳟 (<i>Oncorhynchus mykiss</i>)	[110, 111]
			大西洋鲑 (<i>Salmo salar</i>)	[114]
	<i>tlr4</i>	嗜水气单胞菌、脂多糖	南亚野鲮	[106]
	<i>tlr5</i>	嗜水气单胞菌	南亚野鲮	[106]
	<i>tlr8</i>	鳃弧菌	日本牙鲆	[108]
	<i>tlr9</i>	重组虹鳟鱼肿瘤坏死因子 α 蛋白, 聚胞苷酸	虹鳟	[111]
	<i>tlr9b</i>	鳃弧菌	欧洲鳗鲡	[109]
	<i>tlr20a</i>	鳃弧菌	欧洲鳗鲡	[109]
	<i>tlr21</i>	鳃弧菌	欧洲鳗鲡	[109]
RIG-I样受体(RLR)	<i>rig1</i>	鱼正呼肠孤病毒	大西洋鲑	[114]
	<i>lgs2</i>			
NOD样受体(NLR)	<i>nod1</i>	嗜水气单胞菌、脂多糖	南亚野鲮	[106]
	<i>nod2</i>	嗜水气单胞菌、脂多糖	南亚野鲮	[106]
		病毒性出血性败血症病毒	虹鳟	[112]
	<i>nrx1</i>	病毒性出血性败血症病毒	虹鳟	[112]
	<i>nlrc3</i>	病毒性出血性败血症病毒	虹鳟	[112]
	<i>nlrc5</i>	病毒性出血性败血症病毒	虹鳟	[112]

也可通过上调 *gatal* 和 *scl* 表达而介导其受体 EPOR 上调, 从而提高细胞对 EPO 的敏感性^[104]。

3.2 病原胁迫与感染免疫

长久以来, 结构简单的红细胞仅仅被认为是承担气体运输功能的细胞, 围绕其功能展开的研究大多基于细胞形态和血红蛋白结构等, 其发育和功能缺陷研究更多的是作为人类疾病诊断的辅助判断依据。区别于母体与胎儿间的胎盘屏障使得哺乳动物具有更稳定的胚胎发育环境, 卵膜是鱼类胚胎抵御病原入侵的第一道防线, 在脱膜后, 幼鱼暴露于水体环境而面临了更多病原侵染的风险。尽管胚胎中存在来自母源转移的抗体^[105], 适应性免疫过程仍依赖成熟淋巴细胞的参与, 故鱼类在早期发育过程中, 在其适应性免疫尚未完全建立并发挥作用前, 其更依赖先天免疫来维持体内平衡。

自1981年“红细胞免疫系统”被首次提出以来^[113], 越来越多的研究表明有核红细胞在免疫过程中发挥着重要作用。本综述总结了红细胞在先天免疫中的作用, 其既可作为病原攻击对象导致其分化发育受到病原的调控, 又可发挥近似免疫细胞的作用完成吞噬和抗原呈递等免疫活动。针对病原胁迫下的鱼类红细胞转录组、蛋白组分析中所关注的差异基因涉及病原感知、抗病防御、抗原呈递等复杂免疫过程^[114, 115](表 1)。另外, 近期, 研究人员对人早期胚胎不同发育阶段的有核红细胞进行无偏颇分析发现了具有免疫调控特征的有核红细胞亚群, 开拓了哺乳动物有核红细胞的新功能研究领域^[116]。因此, 研究鱼类有核红细胞与病原胁迫间的相互作用及有核红细胞的免疫调控功能正在成为鱼类抗逆种质选育中的重要研究方向。

病原胁迫下鱼类红细胞免疫响应 病原微生物感染引发病原体与宿主间复杂的免疫反应(图 4)。病原体上存在多种进化保守的特征分子, 如病原体相关分子模式(Pathogen-associated molecular patterns, PAMPs), 其与先天免疫细胞上表达的模式识别受体(Pattern recognition receptors, PRRs)相互作用指示机体识别有害抗原, 进而激活免疫相关信号通路快速诱导炎症反应。常见 PRR 有 Toll 样受体(Toll-like receptors, TLRs)、视黄酸诱导基因 I (RIG-I) 样受体(Retinoic acid-inducible gene I-like receptors, RLRs)、AIM2 样受体(Absent in melanoma 2-like receptors, ALRs)和核苷酸结合受体寡聚化结构域(NOD)样受体(Nucleotide-binding oligomerization domain-like receptors, NLRs)^[117]。目前已有多项在虹鳟(*Oncorhynchus mykiss*)^[118]、大西洋鲑(*Salmo salar*)^[119]等鱼类中的研究证实鱼类红细胞上广泛存在多种 PRR,

表 1 中列举了多项针对鱼类红细胞上 PRR 表达调控的研究。结果表明, 在病原感染发生后, 鱼类红细胞上 PRR 广泛上调, 以激活高水平的机体先天免疫反应。红细胞上不同的 PAMP-PRR 结合模式使鱼类对细菌和病毒感染触发的先天免疫反应调控路径不同^[120]。

除 PAMP 外, 感染导致的大量溶血会将大量红细胞损伤相关分子模式(Damage-associated molecular patterns, DAMPs)如血红素、热休克蛋白等释放至循环中, 激活多种炎症通路导致过度炎症^[121, 122]。在鼠中已开展有许多关于红细胞 DAMP、炎症紊乱与溶血性疾病的相关研究, 但在鱼类中似乎还是空白领域。

在病原感染发生后, 红细胞自身可以表达各种免疫相关基因或分泌细胞因子直接参与机体免疫, 亦可通过调节其他免疫细胞的活性增强整体免疫水平。鱼类先天免疫机制的中心集中于干扰素(Interferon, IFN), 这与哺乳动物中的研究具有一致性。IFN 由病毒与内体或质膜中 TLR 或细胞质受体(主要是 RLR)结合诱导产生, 作用于干扰素受体导致下游信号级联的激活。IFN 导致干扰素刺激基因(Interferon stimulated exonuclease genes, ISGs)转录, ISG 包括黏病毒抗性蛋白(Mx)、2'-5'寡腺苷酸合成酶(OAS)、蛋白激酶 RNA 激活(PKR)、*viperin*、干扰素刺激基因 15 (ISG15)、具有四肽重复序列的 IFN 诱导蛋白(IFIT)和三联基序(TRIM)家族、*tetherin* 等。感染传染性鲑贫血病毒(Infectious salmon anemia virus, ISAv)的大西洋鲑红细胞表现出 IFN- α 基因上调^[123]。VHSV 攻击的虹鳟头肾红细胞表现出与 IFN 信号传导相关基因的显著上调^[112], 用 G-VHSV 质粒 DNA 疫苗体外感染红细胞可触发 *mx* 表达^[124], 而体内感染则显示在红细胞中 Mx、IFIT5 等及抗菌肽 BD1 的表达增加^[112]。虹鳟红细胞在聚胞苷酸刺激下具有特定的转录反应, 分泌可调节巨噬细胞抗病毒反应的热不稳定分子, 表明红细胞可触发白细胞中 PAMP 介导的反应^[125]。

补体系统是先天免疫的核心组成部分, 在免疫系统中发挥免疫细胞激活、趋化性、调理作用和抗原裂解等作用^[126]。鱼类中关于红细胞在补体系统中发挥作用的研究大多关于基于其免疫黏附、吞噬功能、对其他免疫细胞的激活作用等。虹鳟红细胞上高度表达 CRI^[127], 可识别并结合含有补体的免疫复合物, 具有吞噬白色念珠菌并形成玫瑰花结的能力^[128], 这些免疫复合物随血流转运至肝和脾等组织后被吞噬系统清除^[129]。而红细胞自身具有的吞噬作用已经在草鱼(*Ctenopharyngodon*

idella)^[130]、胡子鲇(*Clarias fuscus*)^[131]、虹鳟^[128]等鱼类中被直接观察到,红细胞发挥与巨噬细胞类似的免疫原理,这可能是由于二者均是髓系细胞(髓系广义上包括粒细胞、红细胞、巨核细胞、单核细胞等),具有由髓系祖细胞到髓系细胞发育分化及功能的相似性。

病原胁迫下鱼类红细胞分化调节 红细胞由于携带大量的营养成分及其循环特性进而被多种病原微生物当作攻击的目标,病原微生物感染可能导致红细胞破裂或导致其生成受阻,因此贫血是病原感染引起的最突出的病理生理表现之一^[132]。某些弧菌、气单胞菌、爱德华氏菌、鞭毛虫等致病微生物导致的贫血表征,在鳊^[133]、罗非鱼^[134]、鲑^[135]等多种常见养殖鱼中均可观察到。然而,在病原微生物胁迫背景下,病原微生物直接侵入或毒素引起的红细胞破裂溶血与介导加速清除是一方面,另一方面,感染后环境炎症介质和细胞因子对造血干祖细胞分化和红细胞的生成及其细胞内转录翻译调控产生影响。

正如前文所述,IFN作为鱼类先天免疫中心分子会诱导一种抗病毒状态,宿主细胞倾向于阻断mRNA转录和翻译,以防止病毒在受感染的细胞中复制^[136]。早期,研究人员在成体斑马鱼中注射传染性造血器官坏死病毒(Infectious hematopoietic necrosis virus, IHNV)或传染性胰脏坏死病毒(Infectious pancreatic necrosis virus, IPNV)后,1—2d内可见终末分化红细胞大量减少,因此, IHNV和IPNV

感染可能阻碍造血系统中终末红细胞的分化^[137]。2005年,Phelan等^[138]首次利用蛇头弹状病毒(Snakehead Rhabdovirus, SHRV)对斑马鱼24hpf胚胎进行浸泡感染,实验组幼鱼中可见明显的红细胞减少,而IFN和Mx表达相较于对照组有所增加。在一项体外实验中,提取自金鱼肾脏的祖细胞感染锥虫后,相较于健康组,组内*runx1*、*cmyb*和*gata2*表达明显减少,且*gata1*和*lmo2*的表达也检测出相同趋势,说明寄生虫感染可能阻碍造血祖细胞增殖和维系及其向红细胞的分化^[139],而体内实验则表明感染期间的长期贫血状态可能由IFN γ 增加从而抑制正常红细胞生成引起的^[140]。此外,病原体也可能直接将红系祖细胞作为靶细胞进行侵染,如大西洋鲑中红系祖细胞是PRV进攻的靶细胞^[141]。

4 展望

快速发展过程中产生的资源短缺和水质污染等问题正在促使中国水产养殖从单一追求产量向追求产量和质量并行的转变,如何从渔业养殖中实现经济效益与环境保护的有机统一是育种学家不断探索的重要命题。鉴于鱼类红细胞在其生命活动中承担的重要功能,前文所提到的许多研究已将鱼类红细胞发育和逆境胁迫联系起来。然而,目前在鱼类中开展的研究绝大多数将红细胞作为逆境胁迫下评估鱼类健康的血液指标^[142, 143]。事实上,从鱼类红细胞发育的角度出发实现鱼类抗逆,主要有两种思考方式:(1)创制对逆境不敏感的品种,如

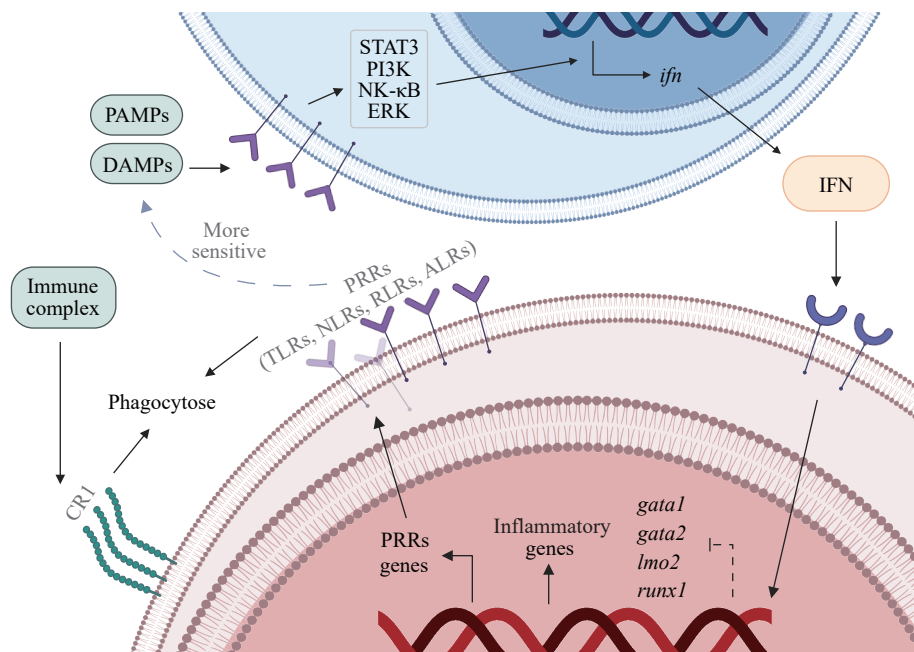


图4 病原胁迫下鱼类红细胞免疫

Fig. 4 Erythrocyte immunity in fish under pathogen stress

正常水平下即拥有高红或高红系分化特征的品种,或红细胞不容易被病原微生物攻击的品种等;(2)创制逆境中红细胞可快速识别病原或响应低氧胁迫的品种。

4.1 基因编辑等应用于鱼类抗逆新种质创制

在低氧胁迫方面,目前已有几项研究阐述了早期红细胞发育与抗低氧性状形成之间的关系^[144, 145]。例如, *hif3a*^{-/-}斑马鱼被发现 *gata1*、*hbae1*、*hbae2*等红系发育调控和标记基因表达下调,红细胞数量减少,表现出不耐低氧胁迫的表型。研究发现, HIF3A 识别并结合位于 *gata1* 启动子上的缺氧反应元件而激活 *gata1* 表达,从而调节红细胞生成^[144],提示基因 *HIF3a* 的功能完整性是鱼类抗低氧等抗逆性状形成的重要遗传基础,其功能缺失将导致鱼类不耐低氧胁迫。斑马鱼 *cox17*^{-/-} 胚胎在其发育早期(60hpf 以前)表现出下调的 Wnt 信号传导和上调的内质网应激(Endoplasmic reticulum stress, ERs), *gata1a*⁺ 细胞中的 β -Catenin 显著减少,并且 TCF4 在 *scl* 和 *lmo2* 启动子上的结合富集显著减少,在胚胎发育早期呈现短暂的红细胞发育缺陷和不耐低氧胁迫^[146]。类似地, *eafl1/2* 缺失导致 TCF4 在 *scl* 和 *lmo2* 启动子上的结合富集显著减少,进而导致 24hpf 时突变体中 *scl* 和 *lmo2* 的表达显著降低,且原始红细胞生成过程中 *eafl1/2* 通过抑制表观遗传修饰蛋白 H3K27me3,而激活 *gata1a* 进而促进红细胞发育^[145]。但基因 *vhl* 的功能缺失鱼类将产生更多的红细胞^[147],并对缺氧环境不敏感。这些研究结果充分提示调控鱼类红细胞发育和生成的重要调控基因和元件是鱼类抗逆性状形成的重要遗传基础的组成。其中,调控红细胞发育和生成的正向遗传因子或表观信号的功能缺失将导致鱼类红细胞生成受阻,不耐低氧,或不能对低氧胁迫做出敏感响应。而调控红细胞发育和生成的负向调控因子的挖掘,将有助于获得更多遗传分子模块,在未来的鱼类优良新种质的创制中,利用调控元件或基因功能缺失编辑技术进而获得产生更多红细胞,或对低氧胁迫不敏感,或快速响应低氧胁迫的鱼类新种质。

在病原胁迫方面,近期一项研究表明,人类受到 SARS-CoV-2 攻击后,血液中的 IFN 反应先于鼻咽反应^[148],说明血液在病原快速响应中的重要作用。鱼类早期发育过程中的病原感染导致的红细胞上模式识别受体、免疫信号上调,提示鱼类红细胞在病原识别、免疫激活上的作用可能比其在吞噬免疫上的作用更高效。一项将红细胞作为 DNA 疫苗诱导目标免疫信号的体外实验^[124],多数已被探明的具有抗病毒功能的基因被发现位于鱼类

IFN 信号通路^[149],而鱼类红细胞在病原胁迫后显示出广泛的 IFN 激活,提示红细胞作为鱼病疫苗免疫的重要靶标以应对病原感染导致的溶血和增殖的研究,是研究红细胞免疫的一个重要研究方向。另外,近期一项研究发现了人早期胚胎具有免疫调控特征的有核红细胞亚群^[116],进一步说明作为终身拥有有核红细胞的鱼类,在研究红细胞与病原胁迫间的相互作用及有核红细胞的免疫调控功能上极具优势。因此,开展鱼类有核红细胞与病原胁迫间的相互作用及其免疫调控功能研究,并深入研究其相互作用过程中的细胞核和各种细胞器的转录调控和代谢反应具有潜在价值。新兴的基因组资源和基因组技术揭示了多种鱼类性状形成的分子机制和关键基因,类似在鱼类肌间刺发育^[150]、饲料转化率^[151]等方面的研究,结合多组学分析手段拓展鱼类红细胞免疫功能研究的新领域,同时挖掘的鱼类中对病原胁迫响应的正向或负向调控因子也将成为关键的候选调控因子可应用于基因编辑,进而创制红细胞不容易被病原微生物攻击的品种,或可快速识别病原并响应的品种。对一些性成熟周期长的鱼类品种,如草鱼和青鱼等,则可利用基因编辑联合借腹怀胎技术^[152],快速高效地创制鱼类新种质。

4.2 传统选育结合分子标记技术应用于鱼类抗逆新种质筛选

传统的养殖鱼类选育是保留生长快、饲料利用率高、耐逆境胁迫、低应激等优良性状的品种作为亲本,并一直延续严格的亲本选育标准而进行世代的繁育。这些养殖和选育的过程,让很多鱼类养殖种群获得大量的适应环境的遗传变异并被保留,这些选择的痕迹广泛出现在多种经济鱼类中^[153, 154]。如在种类最丰富的鲤科鱼类中, *hif1a* 基因在演化中的基因组复制事件被认为是其拥有更强低氧适应能力的原因^[155]。另外,我国四大家鱼中的草鱼(*Ctenopharyngodon idella*)和青鱼(*Mylopharyngodon piceus*),属于大体型淡水鱼,其正向调控身体大小的 PIM 家族基因, *PIM1*、*PIM2*、*PIM3* 都发生了大量的基因扩增^[156]。虹鳟的鱼类群体基因组中也存在大量的与生长、免疫反应等相关的基因组变异和基因组特征等^[157]。生长在高原的人类,为适应其低氧环境,其红细胞数量和血红蛋白含量远超平原地区的居民^[158]。因此推测,一些提升红细胞携氧能力或血红蛋白含量的抗低氧适应性性状的调控分子和一些提升红细胞细菌吞噬能力或细胞因子分泌能力的抗感染性状的调控分子,被选育保留并被世代传递,但有待鱼类分子遗传育种学

家的挖掘。

在未来抗低氧、抗病原感染的鱼类种质选育中,依赖于红细胞发育及其低氧响应的分子调控网络的研究结果而形成的分子和基因模块库,可用于遗传筛选的分子标记并应用于鱼类优良种质的筛选。另外,通过比较基因组分析,结合红细胞发育的分子调控网络研究结果,筛查低氧适应或抗病原感染鱼类中与红细胞发育和生成相关的分子调控元件,并鉴定它们调控鱼类红细胞生成的功能分子机制,同时鉴定它们在抗低氧和抗病原感染中的调控功能,将从红细胞发育和生成的角度夯实传统的鱼类优良种质选育育种技术的遗传基础,并丰富鱼类优良种质筛选的分子标记。

目前已有不少在单一胁迫条件(如低温、高温、低氧、单一病原等)下开展对鱼类红细胞发育调控的研究,但集约环境中的养殖鱼往往同时面临复杂逆境或多种病原感染的威胁,“鱼类-病原体-环境”间的交叉作用及其复杂性可能造成不同的造血尤其是红细胞造血生成的信号响应调节的复杂性^[159],因此在实验室环境中开展鱼类多因子胁迫研究可能更有利于理论机制向育种实践的转化。红细胞发育调控中的适应性表观修饰受外界应激胁迫的影响,其在一定程度上亦可以反映养殖环境的适宜水平,可能为环境因子可视化监测体系开发和“生物安全观”在水产养殖中的系统性工程建设提供理论基础。

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RESEARCH PROGRESS ON EARLY STAGES ERYTHROGENESIS AND STRESS RESISTANCE TRAITS FORMATION IN FISH

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Abstract: The cultivation of new fish species with advantage traits, such as hypoxia tolerance (resistance to abiotic adversity), disease resistance (resistance to biotic adversity), and lower stress responses, is crucial for solving the problems in intensive aquaculture, particularly in facility-based and high-density systems. The hematopoietic system is crucial for maintaining the normal life activities of fish. Erythrocytes, which are involved in the transport of oxygen and nutrients as well as in the immune defense, play vital roles in regulating biological processes related to disease resistance and hypoxia tolerance. By focusing on erythropoiesis in fish, this review elaborates on the physiological functions of fish erythrocytes in terms of oxygen transport and immunity. It highlights the adaptive regulating mechanisms of erythropoiesis under adversity stresses, and focuses on the molecular mechanisms in regulating erythropoiesis. This review discusses the fundamental research on the genetic basis of stress resistance in fish, from the perspective of erythropoiesis development, providing important insights into the theoretical basis for creating new germplasm resources with enhanced stress resistance for aquaculture.

Key words: Hematopoietic system; Erythropoiesis; Red blood cell immunity; Hypoxia tolerance; Disease resistance; Environmental stress; Resistance breeding