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· 综述 ·

硬骨鱼类鳃黏膜免疫相关分子的研究进展

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摘要: 鳃为鱼类重要的呼吸器官, 是鱼类进行离子交换、酸碱调节和含氮废物排泄的重要结构基础, 也是鱼类重要的外周黏膜免疫器官之一, 在抵御病原微生物侵染过程中发挥重要的免疫屏障作用。当前, 硬骨鱼类鳃黏膜免疫反应是研究热点之一。本文首先对硬骨鱼类鳃的结构和特点进行分析, 之后综述了抗菌肽、干扰素、白细胞介素、Toll 样受体、补体等先天性免疫相关分子以及 T 细胞受体和免疫球蛋白等适应性免疫相关分子在硬骨鱼类鳃黏膜中的表达规律、分子功能, 最后探讨了化学因素(重金属、杀虫剂等)、生物因素(细菌、病毒、真菌、和寄生虫等)以及营养物质和疫苗等对硬骨鱼类鳃黏膜结构的影响, 以期深入研究鳃在鱼类黏膜免疫反应中的角色和应答机制提供指导, 为硬骨鱼类病原性疾病的免疫防控策略的制定提供理论基础。

关键词: 硬骨鱼; 鳃; 黏膜免疫; 免疫相关分子

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鳃是硬骨鱼类进行离子交换、酸碱调节和含氮废物排泄的重要器官, 也是鱼类与外界直接接触的黏膜器官。硬骨鱼类鳃表面覆盖黏膜和丰富的黏液, 用以抵御外界刺激^[1]。鳃黏膜组织中免疫细胞丰富, 是鱼类重要的黏膜相关淋巴组织^[2], 在鱼类抵御病原微生物侵袭和感染过程中发挥重要的免疫屏障作用^[3]。本文对硬骨鱼类鳃黏膜免疫相关分子进行了综述, 并探讨了影响鳃黏膜结构的因素, 期望能够为深入研究硬骨鱼类鳃黏膜免疫反应提供指导。

1 鳃的结构及特点

大多数硬骨鱼类鳃包括鳃耙、鳃弓、鳃丝、

鳃小片及鳃盖等结构^[4-5]。鳃耙为锯齿状骨突起, 由表面上皮细胞和基部细胞组成, 具有滤食和保护鳃丝的功能。鳃弓位于鳃丝和鳃耙之间, 是支撑鳃丝和鳃耙的主要骨架。鳃丝呈梳状排列在鳃弓上^[6], 鳃丝表面具有规则或不规则分布的环形微嵴、沟、坑、孔等结构, 鳃丝向两侧伸出形成鳃小片。鳃小皮富含上皮细胞、柱细胞、内皮细胞、黏液细胞、泌氯细胞和毛细血管网, 其中泌氯细胞含有丰富的线粒体和排泄小泡^[5-7], 是行使呼吸功能的基本单位; 黏液细胞则不断产生黏液, 形成一个保护和缓冲鳃细胞免受外部环境的屏障, 污染、极端离子和 pH 值的变化、病原微生物感染及其他应激源会增加黏液细胞数量和黏液分泌^[4]。

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此外, 硬骨鱼的初级鳃丝基部还有不同形状和大小的淋巴上皮。鳃复杂的结构特征与鱼类相应的呼吸功能和体内离子转运、能量代谢、免疫防御等生理功能相呼应^[5]。

2 鱼类鳃黏膜先天性免疫相关分子

鱼类的先天性免疫系统在防御细菌、病毒、真菌、寄生虫等病原生物侵染中发挥着重要作用。目前, 在鱼类鳃中发现了多种天然免疫分子, 如抗菌肽 (antimicrobial peptides, AMPs)、干扰素 (interferons, IFNs)、白细胞介素 (interleukins, ILs)、Toll 样受体 (Toll-like receptors, TLRs)、补体 (complement, C) 等, 可以非特异性杀死或抑制病原微生物的生长繁殖。

2.1 AMPs

抗菌肽又称抗微生物肽或宿主防御肽 (host defence peptides, HDPs), 是一类在自然界广泛存在且具有生物活性的小分子多肽^[8]。AMPs 通过多种机制表现出抗菌、抗真菌和抗寄生虫活性, 包括微生物膜的失稳破坏、孔道形成、蛋白质聚集、抑制细胞内靶点、干扰 DNA 转录以及阻断蛋白质合成和折叠等^[9]。硬骨鱼类鳃黏膜中的 AMPs 主要包括 NK-lysins、 β -defensins、piscidins、hepcidins 等^[8]。

NK-lysins 属于鞘脂激活蛋白样蛋白 (saposin-like proteins, SAPs) 超家族成员, 主要由细胞毒性 T 淋巴细胞 (cytotoxic T lymphocytes, CTL) 和自然杀伤细胞 (natural killer cells, NKs) 合成^[8]。NK-lysins 含有 74~78 个氨基酸残基, 且富含正电荷氨基酸, 具有三维球状结构, 包含 6 个保守的半胱氨酸残基, 形成 3 个二硫键^[10]。哺乳动物 NK-lysins 具有抗菌和抗肿瘤活性。鱼类 NK-lysins 同样具有抗菌功能。多种病原菌, 如迟钝爱德华氏菌 (*Edwardsiella tarda*)、嗜水气单胞菌 (*Aeromonas hydrophila*) 能够显著上调 NK-lysins 或其同系物基因在鳃中的表达^[10-12]。NK-lysins 具有广谱杀菌性, 对大肠杆菌 (*Escherichia coli*)^[10,12-13]、副溶血性弧菌 (*Vibrio parahaemolyticus*)、溶藻弧菌 (*V. alginolyticus*)^[10-11]、哈维氏弧菌 (*V. harveyi*)^[10]、创伤弧菌 (*V. vulnificus*)^[11]、单核细胞增生性李斯特菌 (*Listeria monocytogenes*)^[11]、金黄色葡萄球菌 (*Staphylococcus aureus*)^[12-13]、枯草芽孢杆菌 (*Bacillus subtilis*)、嗜水气单胞菌、迟钝爱德华氏菌等病原菌

具有杀菌活性。此外, 鱼类 NK-lysins 在寄生虫感染过程中同样发挥作用。在感染刺激隐核虫 (*Cryptocaryon irritans*) 的初期, 大黄鱼 (*Larimichthys crocea*) 鳃中 NK-lysin 的基因呈快速上调表达^[13]。

β -defensins 为阳离子 AMPs, 其空间结构由 3 个 β -反平行链组成, 6 个半胱氨酸残基构成的 3 个二硫键对该结构起稳定作用^[14]。鱼类 β -defensins 具有与哺乳动物的 β -defensins 相类似的功能。鲷爱德华菌 (*E. ictaluri*)、溶藻弧菌、无乳链球菌 (*Streptococcus agalactiae*) 等病原菌能够显著上调 β -defensins 在鱼类鳃中的表达^[15-17]。同时, 佩鲁兰新副变形虫 (*Neoparamoeba perurans*) 感染后, 大西洋鲑 (*Salmo salar*) 鳃中 β -defensin-3/4 基因表达显著上调^[18], 提示 β -defensins 在寄生虫感染过程中同样发挥免疫功能。人工合成的 β -defensins 多肽对大肠杆菌、爱德华氏菌、鲁氏耶尔森氏菌 (*Yersinia ruckeri*)、嗜水气单胞菌、维氏气单胞菌 (*A. veronii*) 等革兰氏阴性细菌以及金黄色葡萄球菌、海豚链球菌 (*S. iniae*)、无乳链球菌等革兰氏阳性菌均有较高的杀菌活性^[18], 提示 β -defensins 具有开发成渔用抗菌药的潜力。

Piscidin 家族是硬骨鱼特有的两亲性阳离子 AMPs, 包括 piscidin、pleurocidin、moronecidin、misgurin、epinecidin、gaduscidin 和 dicentracin 等亚型^[8], 主要由巨噬细胞、单核细胞、粒细胞、肥大细胞和上皮细胞合成^[19]。Piscidin 家族成员的 N 端高度保守, 含有 1 个组氨酸和 2 个精氨酸, 其 C 端常呈 α -螺旋结构^[20]。溶藻弧菌^[21]、副溶血性弧菌、嗜水气单胞菌以及刺激隐核虫等病原微生物能够上调鳃中 piscidin 家族成员的表达^[22-25]。研究发现, 体外重组大黄鱼 piscidin 可使刺激隐核虫大核肿胀、细胞膜破裂和内容物泄漏, 以及滋养体细胞膜孔隙率、破裂和外排的含量^[22], 提示鱼类 piscidin 在抗寄生虫方面具有很大的应用潜力。此外, 其他病原相关分子模式 (pathogen-associated molecular patterns, PAMPs), 如聚肌胞苷酸 (polynosinic-polycytidylic acid, polyI:C)、 β -葡聚糖 (β -glucan) 也能够上调 piscidin 在鳃中的表达, 提示 piscidin 在多种病原入侵过程中均发挥防御作用^[26]。

鳃中的 hepcidins (又称铁调素) 和 cathelicidins 具有广谱抗菌活性, 二者的合成肽对革兰氏阴性细菌如气单胞菌、灿烂弧菌 (*V. splendidus*) 和革兰氏阳性细菌如金黄色葡萄球菌均具有杀菌作用^[27-28]。此外, 寄生虫 (如佩鲁兰新副变形虫) 或

者 PAMPs(如 β -glucan) 能够显著上调二者在鳃中的表达^[18, 26]。

由此可见, 抗菌肽是鱼类鳃黏膜中重要的先天免疫分子, 对多种病原微生物具有抑制或直接杀伤作用。抗菌肽有望成为新型免疫增强剂用于预防和治疗鱼类相关病原性疾病。

2.2 IFNs

干扰素是一类具有抗病毒增殖和免疫调节活性的细胞因子^[29]。哺乳动物含有 3 种类型的 IFNs, 分别命名为 I 型、II 型和 III 型 IFNs^[30]。鱼类中则也含有 3 中类型的 IFNs, 分别为 I 型、II 型和 IV 型 IFNs (IFN- ν)^[29, 31]。

哺乳类 I 型 IFNs 包括 α 、 β 、 κ 、 ϵ 、 ω/τ 及 δ/ζ 等多个基因亚群。根据半胱氨酸组成的不同, 鱼类 I 型 IFNs 可以分为 4 个亚群, 分别为 I 类亚群 (IFN α 、IFN δ 、IFN ϵ 和 IFN η)(含有 2 个半胱氨酸)、II 类亚群 (IFN β 和 IFN γ)(含有 1 个或 2 个二硫键) 和 III 类亚群 (IFN τ)(含有 1 个或 2 个二硫键)^[30-32]。鱼类 I 型 IFNs 具有与哺乳动物 I 型和 III 型 IFNs 相似的抗病毒活性, 在病毒感染或 PAMPs (如 polyI:C) 刺激后能够显著上调表达^[33-35]。研究发现, 过表达鳃 (*Siniperca chuatsi*) *ifnc* 和 *ifnh* 能够增强 *stat1* 和 *stat2* 磷酸化并诱导干扰素刺激基因 (IFN-stimulated genes, ISGs) 的表达^[35], 提示鱼类 I 型 IFNs 和哺乳类 I 型 IFNs 具有相同的信号传导通路。

哺乳类 II 型 IFNs 只含有 IFN- γ 1 个成员, 而硬骨鱼含有 *ifn- γ* 和 *ifn- γ* 相关 (*ifn- γ rel*) 基因 2 个 II 型 IFNs。*ifn- γ* 主要由活化的免疫细胞产生, 如先天性免疫应答过程中的自然杀伤 (NK) 细胞、巨噬细胞、NKT 细胞和树突状细胞等, 以及适应性免疫过程中的 CD4⁺ T 辅助因子 1、CD8⁺ 细胞毒 T 淋巴细胞效应因子 T 细胞等^[36]。多种病原, 如赤点石斑鱼神经坏死病毒 (Red spotted grouper nervous necrosis virus, RGNNV)、多子小瓜虫 (*Ichthyophthirius multifiliis*)、迟钝爱德华氏菌等均能够显著上调鱼类 II 型 IFNs 在鳃中的表达^[36-40]。此外, PAMPs (如 LPS、polyI:C) 也能够上调鱼类 *ifn- γ* 在鳃中的表达^[37]。这些研究提示, 鱼类 II 型 IFNs 在病毒、细菌及寄生虫感染过程中均发挥重要的免疫功能。过表达黑棘鲷 (*Acanthopagrus schlegelii*) *ifn- γ* 可诱导 JAK-STAT 信号通路相关基因 (*stat1*、*stat2* 和 *irf9*) 及抗病毒基因 *mx1* 和 *isg15* 的表达,

提示鱼类 II 型 IFNs 也是通过 JAK-STAT 通路进行信号转导^[36]。

2.3 白细胞介素

白细胞介素是参与免疫系统细胞间调节的一类细胞因子, 主要由 CD4⁺T 细胞、巨噬细胞、单核细胞和内皮细胞等产生。鳃中高表达的白细胞介素主要有 IL-1、IL-2、IL-6、IL-10、IL-17 及异二聚体 ILs 等亚家族, 其中 IL-1 亚家族属于 β -三叶草型细胞因子, IL-2、IL-6 亚家族属于 I 型 α 螺旋细胞因子, 而 IL-10 属于 II 型 α 螺旋细胞因子, IL-17 则属于半胱氨酸结细胞因子^[41]。

鱼类 IL-1 亚家族含有 IL-1 β 和 IL-18 两个成员^[41]。与哺乳动物 IL-1 β 类似, 鱼类 IL-1 β 也是重要的促炎细胞因子^[41]。细菌、病毒和寄生虫等病原均能诱导 IL-1 β 在鳃中的表达, 但反应时效不尽相同, 如卵形鲳鲹 (*Trachinotus ovatus*) 鳃中 IL-1 β 在哈维氏弧菌刺激 6 h 后显著上调, 在无乳链球菌刺激后 9 h 显著上调, 而在毒性神经坏死病毒 (viral nervous necrosis virus, VNNV) 和刺激隐核虫感染后 1 d 才呈显著上调^[42]。鱼类 IL-18 在细菌感染后同样上调表达^[43]。综述表明, 鱼类 IL-1 亚家族在病原微生物感染中具有重要的免疫调节功能。

鱼类 IL-2 亚家族主要包括 IL-2、IL-4/13、IL-7、IL-15 和 IL-21 等成员^[41]。研究表明, 细菌、病毒和寄生虫感染均能上调 IL-2 亚家族成员在鳃中的表达。迟钝爱德华氏菌和牙鲆弹状病毒 (hirame novirhabdovirus, HIRRV) 感染能够显著上调 IL-2 在鳃中的表达^[44]。佩鲁兰新副变形虫感染后大西洋鲑鳃 IL-4/13 表达显著上调^[45]。多子小瓜虫感染后虹鳟 (*Oncorhynchus mykiss*) 鳃中 IL-4/13 的相对表达量显著提高^[46]。研究发现, 斑马鱼 (*Danio rerio*) IL-4/13 参与维持鳃中 Th2 样表型和抑制炎症免疫反应^[47]。此外, IL-2 在鳃部免疫细胞增殖方面也发挥作用。冷冻切片和间接免疫荧光测定显示体内注射重组 IL-2 蛋白促进了牙鲆 (*Paralichthys olivaceus*) 鳃中 CD4⁺ 和 IL-2R β ⁺ 细胞的增殖, 流式细胞仪分析显示体外用 IL-2 蛋白处理后 CD4⁺ T 淋巴细胞和 IL-2R β ⁺ 细胞也显著增加^[48]。

其他鳃中高表达的 ILs 在同一病原或不同病原感染具有时效差异性。刺激隐核虫感染后卵形鲳鲹鳃中的 IL-11 无显著变化, 而 IL-34 则在感染后 6 h 时开始显著上调; VNNV 感染 7 d 后卵形鲳鲹鳃中的 IL-11 出现上调表达, 而 IL-34 则在感染

后 1 d 和 7 d 显著上调^[42]。肺炎克雷伯氏菌 (*Klebsiella pneumoniae*) 感染后, 尖嘴鳄 (*Lepisosteus oculatus*) 鳃中 *IL-8* 显著上调, 而 *IL-10* 的表达则显著下调^[43]。由此可见, 不同的 ILs 在硬骨鱼鳃部抵御病原侵袭过程中具有明显的时效性差异, 这可能与不同 ILs 具有不同的免疫效应有关。

2.4 Toll 样受体

Toll 样受体是一类保守的能够识别 PAMPs 并诱导免疫效应分子的模式识别受体 (patterns recognition receptors, PRRs), 在机体的先天性免疫反应以及适应性免疫激活过程中发挥重要作用^[49]。哺乳类和鱼类 TLRs 具有类似结构, 均包含一个富含亮氨酸重复区 (leucine-rich repeat, LRR) 的胞外区、一个跨膜区和一个带有 Toll/IL-1 受体域 (Toll/interleukin-1 receptor, TIR) 的胞内区^[49]。哺乳类中已经发现 13 个 TLRs (TLR1~TLR13), 鱼类中则发现了至少 20 个 TLRs^[50]。鱼类除了含有大部分哺乳类 TLRs 外 (缺失 TLR6 和 TLR10), 还含有鱼类所特有的 TLRs, 包括 TLR5S、TLR18~TLR20, TLR22~TLR28^[51]。目前, 对于鱼类大多数 TLRs 的配体仍有待确定。在鳃中高表达的 TLRs 主要有 TLR1~TLR4、TLR7、TLR9、TLR12、TLR18、TLR21~TLR22。但是, 目前对于 TLRs 在鳃部的免疫功能研究相对较少, 且多以基因表达为主。在细菌感染、寄生虫感染或 PAMPs 刺激能够显著上调鳃 TLRs 基因表达。LPS 刺激大菱鲆 (*Scophthalmus maximus*) 后, 其鳃中 *tlr2* 在刺激后第 4 天开始显著上调, 而 polyI:C 刺激大菱鲆后 *tlr2* 则在第 2 天就开始显著上调, 表明鳃中的 *tlr2* 在应对 RNA 病毒感染更为迅速^[52]。多子小瓜虫感染后斑点叉尾鲷 (*Ictalurus punctatus*) 鳃中 *tlr1* 和 *tlr9* 亦显著上调表达^[39]。上述研究表明, 鳃 TLRs 在鱼类抵御病原微生物入侵中发挥作用, 但确切的识别配体以及信号通路有待深入研究。此外, 在污染物或环境胁迫下, 鳃 TLRs 的表达也会发生变化。氯化三丁锡急性和慢性刺激后, 暗纹东方鲀 (*Takifugu obscurus*) 鳃中 *tlr18* 和 *tlr22* 基因表达水平显著上调, 而鳃中 *tlr2* 和 *tlr3* 显著下调^[53]。急性缺氧使花斑裸鲤 (*Gymnocephalus eckloni*) 鳃中 *tlr1* 的表达显著上调, 而 *tlr3*、*tlr4*、*tlr7* 和 *tlr12* 等基因表达显著下调^[54]。上述研究表明, 鳃部 TLRs 在机体组织损伤以及生理变化过程中同样发挥作用。

2.5 补体

补体系统由超过 35 个血清蛋白组成, 在机

体的先天性免疫和适应性免疫中均发挥重要作用^[9]。补体的激活途径包括经典激活途径、旁路激活途径和凝集素途径, 3 条激活途径最终形成膜攻击复合物 (membrane attack complex, MAC)。在经典补体激活途径中, 抗原抗体复合物与 C1q 结合, 导致 2 个 C1r 相互活化, C1r 随后活化 C1s, 活化的 C1s 裂解 C4 为 C4a 和 C4b 片段, C4b 结合到靶细胞膜上并在膜上与 C2 结合, 随后 C2 也被 C1s 裂解为 C2a 和 C2b。C2a 和 C2b 在细胞表面形成 C4b2a 复合物, 即 C3 转化酶, 该酶将 C3 裂解为 C3a 和 C3b。C3b 与细胞膜上的 C4b2a 结合形成 C4b2a3b 复合体, 即 C5 转化酶。在旁路激活途径中, 细菌的细胞壁成分如 LPS、肽聚糖 (peptidoglycan, PGN) 存在时, C3b 结合到细胞表面并与 B 因子结合, B 因子又被 D 因子切割而在细胞表面形成 C3bBb 复合体, 即旁路途径中的 C3 转化酶, 该酶可以活化更多的 C3 参与旁路途径。在凝集素途径中, 甘露糖结合凝集素 (mannose-binding lectin, MBL) 或 ficolin 与微生物表面结合, 之后通过 MBL 相关丝氨酸蛋白酶 (MBL-associated serine protease, MASP) 活化 C4 和 C2, 然后进入与经典途径相同的 C3 转化酶和 C5 转化酶过程。上述 3 个途径形成的 C5 转化酶裂解 C5 分子为 C5a 和 C5b 片段, C5b 与 C6 和 C7 结合而形成 C5b67 复合体。该复合体在细胞膜上与 C6-C9 形成 MAC, 最终导致细胞膜穿孔, 细胞内容物外渗出, 引发细胞死亡^[55-56]。

鱼类具有与哺乳类相似的补体分子。这些补体分子在鳃组织高表达, 并在病原微生物感染过程中呈显著上调表达。许氏平鲷 (*Sebastes schlegelii*) 感染鳃弧菌 (*V. anguillarum*) 后 *c1s*、*c8b*、*c8g* 在鳃中呈显著上调表达^[57]。泥鳅 (*Misgurnus anguillicaudatus*) 感染嗜水气单胞菌 36 h 后 *c3*、*c4*、*c1qb*、*c2b*、*c8b* 在鳃中也显著上调表达^[58], 而草鱼 (*Ctenopharyngodon idella*) 感染嗜水气单胞菌后 4 h 鳃中的 *c3* mRNA 开始上调表达^[59]。上述研究表明, 鳃组织补体在应对病原微生物侵染过程中存在一定的时序性。而且, 多子小瓜虫、刺激隐核虫等寄生虫亦可显著诱导鳃组织的补体基因的表达^[38,60-61], 提示鳃组织补体在不同的病原微生物感染中均发挥免疫作用。过表达草鱼 C4 能显著提高 *IL-1 β* 、*tnf- α* 等基因的表达, 提示鱼类补体系统在鱼类的炎症反应过程中同样发挥作用^[62]。病原微生物会通过干扰补体系统的激活而入侵鱼体。

嗜水气单胞菌可以降解草鱼 C3, 并抑制 MAC 的形成而逃过补体系统对其的杀灭^[63]。当前, 对于鱼类补体系统的激活与调控是研究的热点。Yu 等^[64]在松江鲈 (*Trachidermus fasciatus*) 中克隆鉴定了一个新的 MBL 同源基因 CL-11, 可以结合 MASP 并提高 MAC 的水平。Mu 等^[65]在尼罗罗非鱼 (*Oreochromis niloticus*) 中克隆鉴定了 MBL 相关蛋白 34 (MBL associated protein, MAP34), 其可以竞争 MASP 与 MBL 的结合而抑制凝集素途径的激活。

3 鱼类鳃黏膜适应性免疫相关分子

鱼类是同时拥有先天性免疫和适应性免疫的低等脊椎动物。适应性免疫应答包括 T 细胞介导的细胞免疫和 B 细胞介导的体液免疫应答, 负责清除感染后期的病原体, 并对特定抗原产生免疫记忆^[66]。鳃组织富含 T 细胞和 B 细胞, 其适应性免疫相关分子主要有 T 细胞受体 (T cell receptors, TCRs) 和免疫球蛋白 (immunoglobulins, Igs) 等。

3.1 TCRs 相关分子

TCRs 是 T 细胞表面的特征标志, 由 T 淋巴细胞等产生, 是先天性免疫和适应性免疫系统之间的桥梁, 负责识别和呈递外来抗原。哺乳动物有 4 种 TCRs, 分别是 TCR α 、TCR β 、TCR γ 和 TCR δ , 其中 TCR α 、TCR β 识别与 MHC 复合体分子结合的肽段; TCR γ 和 TCR δ 直接识别抗原。根据 T 细胞表达 TCRs 的不同, 成熟的 T 细胞可以分为 2 种亚型: $\alpha\beta$ -T 细胞和 $\gamma\delta$ 细胞。鱼类中也含有 TCR α 、TCR β 、TCR γ 和 TCR δ 4 种 TCRs, 且 T 细胞也可分为 $\alpha\beta$ -T 细胞和 $\gamma\delta$ 细胞^[67]。研究发现, 细菌、寄生虫等感染均能诱导鳃组织 TCRs 的表达。鳃 TCR α 在灭活柱状黄杆菌 (*Flavobacterium columnare*) 刺激后 2 周显著上调表达, 而其 TCR γ 则在感染后 4 周呈上调表达^[68]。泥鳅 TCR α 和 TCR β 在柱状黄杆菌感染后 4~21 d 均呈显著上调表达^[69], 而 TCR γ 和 TCR δ 仅在感染后第 4 天显著上调表达^[70]。此外, 多子小瓜虫、水霉等亦能上调鱼类 TCR 在鳃组织中的表达^[69-70]。上述研究表明, 鱼类鳃组织 TCRs 在病原微生物侵染中发挥重要作用。

鱼鳃中的 T 淋巴细胞表面还具有 CD4 或 CD8 等表面标记。研究发现, 鳃中 CD4-1⁺ T 细胞数量要比 CD8 α ⁺ T 细胞的数量多^[71]。不同浓度鲑贫血

病毒 (infectious salmon anaemia virus, ISAV) 感染大西洋鲑 (*Salmo salar*) 后, 相比高浓度组, 低浓度组鳃细丝底部的上皮细胞中有更多 CD8 α ⁺ T 细胞^[72], 表明高浓度 ISAV 感染可能消耗了 CD8 α ⁺ T 细胞。此外, 多子小瓜虫感染的虹鳟鳃丝基部 CD8 α ⁺ T 细胞浸润^[38]。鱼类中的 CD8⁺ T 细胞与哺乳类细胞毒性 T 细胞的功能相类似^[73]。上述研究表明, 鱼鳃在防御病原体入侵中发挥重要作用。

3.2 免疫球蛋白

免疫球蛋白是 B 细胞介导的体液免疫应答反应的主要成分。分泌性的 Igs, 即抗体, 在中和抗原及病原清除中发挥关键作用。膜结合型 Igs 则是 B 细胞表面标记物。目前, 在鱼类中已经发现 3 种 Igs, 分别为 IgM、IgD 和 IgZ/T^[74-75]。鳃黏膜中 IgM 主要以四聚体形式存在, 而 IgD 则以单体形式存在, 二者主要负责识别、结合特异性抗原^[76-77]。IgT 则以单体或聚合物的形式存在于血清或鳃黏液中^[76-77], 主要参与黏膜免疫反应。鳃黏膜 Igs 能够被多种病原诱导表达。柱状黄杆菌感染能够上调虹鳟鳃黏膜中 *igm* 的表达^[78]。多子小瓜虫感染能够上调虹鳟^[38] 和斑马鱼^[48] 鳃中 *igm* 的表达。嗜水气单胞菌、弹状病毒 (rhabdovirus) 及鱼虱 (argulus) 感染能够上调露斯塔野鲮 (*Labeo rohita*) 鳃中 *igd* 的表达^[79]。柱状黄杆菌和多子小瓜虫感染显著上调虹鳟鳃黏膜中 *igt* 的表达并促进 IgT⁺ B 细胞的产生^[76, 78]。柱状黄杆菌和水霉感染同样能够上调泥鳅鳃黏膜中 *igt* 的表达^[80]。IgT 是鱼类中黏膜免疫的重要 Igs, 除了在黏膜部分识别特定抗原外, IgT 在共生菌群稳态中同样发挥作用。虹鳟 IgT 缺乏导致鳃共生微生物群的失调、炎症、组织损伤, 并对寄生虫感染的抵抗力减弱^[81]。

综上所述, 先天性免疫因子和适应性免疫因子在鳃黏膜抵御病原微生物侵袭过程中发挥重要作用, 是鳃黏膜免疫屏障的重要组成部分, 在预防鱼类病原性疾病过程中至关重要。

4 影响鳃结构的因素

鳃是抵御病原感染的重要免疫应答器官, 同时也是病原体侵染的重要门户。保持鳃结构和功能的完整性是保证鱼类健康的重要前提。影响鳃结构的因素主要有化学因素、生物因素、营养物质和疫苗等。

4.1 化学因素

引起鳃损伤的化学因素主要包括重金属(铜、砷、汞等)、杀虫剂等有毒物质。水性纳米铜和硫酸铜会导致洛氏鳃(*Rhynchocypris lagowskii*)^[82]鳃组织增生、动脉瘤和鳃丝坏死,杯状细胞肿胀,黏膜层坏死,引起鱼类氧化应激、组织损伤和渗透调节失衡。过量亚砷酸盐造成斑马鱼鳃组织黏液丢失和上皮细胞表面脱屑等病理学损伤^[83]。砷暴露会导致尼罗罗非鱼鳃上皮增生、隆起和水肿、板层融合、动脉瘤、脱屑和坏死^[84]。低浓度汞暴露会导致黄鳍棘鲷(*Acanthopagrus latus*)鳃黏液分泌增多、细丝上有碎屑和血斑、部分细丝丢失或缩短,层状上皮的广泛提升和层状上皮下面空间扩大的层状水肿、层状上皮脱落、上皮细胞的毛细血管扩张、肥大和增生导致次生层和水空间减少,鳃片变粗、血液充血;高浓度则导致鳃部层状动脉瘤和层状上皮破裂出血^[85]。氯吡硫磷(毒死蜱)可导致攀鲈(*Anabas testudineus*)鳃组织次级薄片的融合和上皮增生、肥大、层状上皮抬高、动脉瘤、坏死和上皮细胞脱屑,氯化细胞中线粒体的肿胀、管状系统的扩张,过多的黏液沉积和核异常^[86]。 λ -三氯氟氰菊酯导致尼罗罗非鱼鳃血管系统充血、嗜酸性粒细胞浸润、黏液细胞增生,次级板层部分融合到血管动脉瘤形成以及板层上皮坏死^[87];氯氰菊酯导致斑马鱼鳃组织细胞的DNA损伤和氧化应激,改变负责维持活性氧平衡的酶的活性及其相应基因的表达^[88]。

4.2 生物因素

引起鳃损伤的生物因素主要有细菌、病毒、真菌、和寄生虫。受柱状黄杆菌感染后,虹鳟幼鱼的鳃肿胀和出血、鳃坏死、次生鳃片融合、肥大、增生和上皮提升^[89]。嗜水气单胞菌感染导致黄鳍棘鲷鳃上皮细胞肥大和增生、板层融合、鳃片呈棒状,上皮隆起,血液充血,黏膜细胞肥大增生,严重者导致鳃组织动脉瘤和充血出血^[90]。杀鲑气单胞菌(*A. salmonicida*)感染后鲤(*Cyprinus carpio*)鳃部板层融合,伴有白细胞浸润、毛细血管扩张和增生层状上皮细胞^[91];鲫(*Carassius auratus*)鳃部出现多灶性坏死和炎性细胞浸润^[92]。鲑鳃痘病毒(salmon gill pox virus)感染造成大西洋鲑鳃上皮细胞凋亡和存活鱼鳃上皮细胞增生,造成鳃呼吸表面损伤^[93],损害黏膜防御。异育银鲫(*C. auratus gibelio*)感染CyHV-2时出现鳃苍白、鳃

组织坏死、鳃周围有点状和瘀斑状出血,鳃裸露、剥落、坏死;鳃板层卷曲、增生、板层融合和充血损伤,局部区域鳃板末端形成血块,鳃次级板层上皮弥漫性肥大和增生,鳃功能丧失^[94]等,大小鱼类死亡率增加。鲤疱疹病毒Ⅲ型(cyprinid herpesvirus 3, CyHV-3)可导致鲤鳃坏死,鳃次生薄片与坏死细胞广泛融合,染色质边缘化,鳃组织中形成核内包涵体^[95],鳃片缺失和鳃炎症细胞浸润,鳃弓的上皮炎症和血管充血增加,感染几天后鳃结构消失,鳃耙的表面上皮细胞局部脱落^[96],严重导致鱼类死亡。鲤春病毒血症病毒(spring viraemia of carp virus, SVCV)感染后造成鲤科(Cyprinidae)鱼类鳃片退化、鳃部点状出血等^[97]。水霉属(*Saprolegnia* spp.)感染鲤会导致初级鳃丝严重出血和水肿,上皮细胞增生、次级鳃丝浸润、炎性细胞浸润和坏死水肿,鳃部呈现棉絮状等症状,血管舒张和充血,鳃继发层融合、鳃层肥大、侵蚀、继发层卷曲、动脉瘤等症状,导致鱼类大量死亡^[98]。黏孢子虫(*Henneguya ictaluri*)会导致鱼体出现增殖性鳃病,引发鳃炎,损害鱼鳃的渗透调节、呼吸和免疫屏障功能^[99-100];佩鲁兰新副变形虫侵袭鱼类时会使鱼类产生阿米巴鳃病(amoebic gill disease, AGD),致使鳃上皮增生、水肿和板层融合,最终死亡^[101]。

4.3 营养物质和疫苗

在鱼类生长过程中,饲喂适量的各种营养物质如蛋白质、氨基酸、维生素对鱼类生长发育、免疫力和抗病力等具有较好的促进作用。日粮蛋白质的最佳添加水平使草鱼鳃中产生的抗菌成分增加,抗炎细胞因子等的mRNA水平上调,而促炎细胞因子、*nf- κ b* P65/P52等的mRNA水平下调,抗氧化酶和谷胱甘肽的活性和mRNA水平升高,活性氧和丙二醛含量降低,NF-E2相关因子2、B细胞淋巴瘤蛋白-2、凋亡抑制蛋白和紧密连接复合物等的mRNA水平上调,半胱氨酸天冬氨酸-蛋白酶3/8/9、凋亡蛋白酶激活因子-1、草鱼鳃中Bcl-2相关X蛋白、肌球蛋白轻链激酶和p38丝裂原活化蛋白激酶等的mRNA水平均下调,同时降低柱状黄杆菌引起的鳃腐病发病率^[5]。草鱼膳食氨基酸、维生素等缺乏或过量可下调紧密连接蛋白的表达,上调促炎细胞因子基因(*IL-1 β* 、*IL-8*、*tnf- α*)的表达,下调抗炎因子(*IL-10*和*tgf- β 1*)的表达,致使鱼鳃结构损伤^[102-104]。同时导致鱼鳃组织

细胞 DNA 片段化、抗氧化防御受损, 适当补充这些营养物质可逆转这些负效应。

此外, 研究表明疫苗对鱼类鳃黏膜免疫功能有积极的调控作用。经鳃给用鳗弧菌疫苗可大大降低鱼体的应激反应, 并显著上调鳃中免疫因子基因 (如 *tlr 4/tlr5/tlr20/tlr22*) 的表达^[105-106]。β-葡聚糖或山萆苣碱作为浸泡免疫佐剂可提高 CyHV-2 疫苗的免疫保护效率, 增强异育银鲫鳃黏膜免疫功能, 减轻鳃组织结构损伤等^[100]。表明适量添加日粮中各种营养物质可以提高鱼鳃的免疫功能、抗病力及物理屏障的功能。

5 展望

鳃是鱼类抵抗病原微生物入侵的黏膜免疫器官。鳃结构的完整性是保证鳃行使各项生理和免疫功能的重要基础。鳃组织免疫相关细胞、免疫分子以及影响鳃结构的因素已有研究, 但鳃黏膜免疫分子调控网络及抗病机制尚不明了。对病原如何突破鳃黏膜屏障及其进一步侵染也知之甚少。此外, 众多因素如何通过鳃黏膜组织影响鱼体的机制也有待探讨。进一步探究鳃黏膜相关免疫细胞和免疫分子的作用机制, 对于研发新型动保产品和疫苗、靶向治疗各种鱼类疾病、降低养殖经济成本和提高养殖效益具有重要的现实意义。

(作者声明本文无实际或潜在的利益冲突)

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Progress in research of the immune-related molecules in gill mucosa of teleost fish

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Abstract: Gill is not only an important respiratory organ of teleost, but also a structural foundation for ion exchange, acid-base regulation and nitrogenous waste excretion of fish. Importantly, it is one of peripheral mucosal immune organs of fish. The mucosal immune response of gill is the hot research topic. In this paper, we firstly analyzed the structure and features of gill. Then, we summarized the gene expressions and molecular functions of molecules involved in the innate immunity and adaptive immunity in gill. Antimicrobial peptides (AMPs), including NK-lysins, β -defensins, piscidins and hepcidins, are important innate immune molecules in fish gill, which can directly inhibit or kill a variety of pathogenic microorganisms. Fish type I interferon (IFN) and type II IFN in gill can be up-regulated by bacteria, viruses and parasites, indicating their important roles in gill immune response against pathogens. The interleukin (IL)-1, IL-2, IL-6, IL-10, IL-17 and heterodimer ILs are mainly expressed in gill, but possess distinct time-dependent patterns following pathogen invasion. Toll-like receptors (TLRs) are important pattern recognition receptors of fish. The TLR1-4, TLR7, TLR9, TLR12, TLR18, TLR21-22 are highly expressed in gill and up-regulated by pathogens. Fish possess similar complement molecules as mammals, and these complement molecules are highly expressed in gill and significantly up-regulated during pathogen invasion. T-cell receptors (TCRs) and immunoglobulins are the main adaptive immune molecules in gill. Similar to mammals, fish have four types of TCRs (TCR α , TCR β , TCR γ and TCR δ). These four TCRs are induced by bacteria or parasites in gill. Three types of immunoglobulins (IgM, IgD, and IgZ/T) exist in gill. IgM and IgD in gill are involved in the recognition and binding of specific antigens, while IgZ/T is responsible for the mucosal immune response. Lastly, the effects of chemical factors (such as heavy metals, pesticides), biotic factor (such as bacteria, virus, parasites), nutriments, and vaccines on the structures of gill were analyzed. Heavy metals (such as copper, arsenic, mercury), pesticides, and pathogens invasion can destroy the normal structure of gills. Nutriments and vaccines have positive regulatory effects on the immune function of fish gill. This study may provide guidance for further study of the functional role and response mechanism of gill in fish mucosal immune response, and provide theoretical basis for the research and development of immune prevention and control strategies for pathogenic diseases of teleost.

Key words: teleost; gill; mucosal immunity; immune-related molecules

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