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Hans汉斯

斑马鱼模型用于人类慢性病研究进展

梁　钰，吴　亮

江苏大学医学院医学检验系，江苏　镇江

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摘　要

慢性病是一类长期发展并持续存在的疾病，主要包括心血管疾病、糖尿病、肿瘤以及神经系统疾病等。随着近年来人口老龄化的加剧，慢性病给个体健康和社会经济带来了巨大的负担和挑战，而开展各种慢性病发病机制和新型治疗方法的研究显得至关重要。斑马鱼是一种模式生物，近年来开始用于遗传发育生物学、人类疾病、环境毒理学和药理学等领域的研究。本文归纳了斑马鱼模型在糖尿病、多发性骨髓瘤和神经系统疾病研究中的进展。

关键词

斑马鱼，模式生物，糖尿病，多发性骨髓瘤，神经系统疾病

Research Progress of Zebrafish Models for Human Chronic Diseases

Yu Liang, Liang Wu

Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang Jiangsu

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Abstract

Chronic diseases are a class of long-term development and persistent diseases, mainly including cardiovascular diseases, diabetes, tumors and neurological diseases. With the increase of population aging in recent years, chronic diseases have brought huge burden and challenge to individual health and social economy, and it is very important to carry out research on the pathogenesis of various chronic diseases and new treatment methods. Zebrafish is a model organism that has been used in recent years in the fields of genetic developmental biology, human disease, environmental toxicology and pharmacology. This paper summarizes the progress of zebrafish model in the research of diabetes, multiple myeloma and nervous system diseases.

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Keywords

Zebrafish, Model Organism, Diabetes Mellitus, Multiple Myeloma, Nervous System Disease

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1. 引言

慢性病是起病隐匿、病因复杂不明、病程较长且久治不愈的疾病总称，包括但不限于各种病症，例如心血管疾病、慢性肺病、癌症、肥胖症、糖尿病、肾脏或肝脏疾病，也涉及慢性感染，例如 HIV 和以炎症性肠病为代表的慢性炎症即克罗恩病和溃疡性结肠炎。如今，慢性病是世界各地的一个主要公共卫生问题，随着人口的增长和老龄化，该问题正在迅速加剧[1] [2]。对慢性病的发病机制、特效药开发和治疗手段的研究刻不容缓。

斑马鱼一直被视作研究发育生物学的模式生物。目前已经在斑马鱼体内发现了一些与哺乳动物类似的常见重要发育机制。斑马鱼个体小、胚胎透明，为镜下活体观察提供了独特条件，且其繁殖能力强、世代间隔短、体外受精操作简单利于得到相同亲本的胚胎。结合遗传操作，包括使用吗啡肽、突变体或转基因鱼系的基因沉默技术，斑马鱼已经成为发育生物学最重要的模型之一。除此之外，斑马鱼也是几种与人类疾病相关的病理生理现象的既定模式生物。例如，斑马鱼因其能够部分补偿器官损失(如心脏和鳍损伤)而被用于炎症和再生研究。它还被用于药物筛选、肿瘤生物学、系统生物学、先天遗传性疾病以及感染研究[2]。本综述总结归纳了斑马鱼在几类人类慢性病中的研究进展。

2. 斑马鱼在糖尿病并发症中研究

斑马鱼胰腺的发育和功能与哺乳动物相似。目前，对斑马鱼体内葡萄糖代谢调节机制的研究发现在斑马鱼发育早期其体内无法检测到葡萄糖，但在受精后 24 h 葡萄糖浓度达到峰值，并在胚胎发育的未来 2 天内迅速下降[3]。成年斑马鱼体内血糖水平约为 50~75 mg/dL，接近人类 100 mg/dL 的生理水平[3] [4]。斑马鱼糖尿病模型有多种诱导方式，如控制斑马鱼进食[3]，显微注射 0.3%链脲佐菌素 350 mg/kg 破坏成年斑马鱼胰腺(血糖浓度升高至 300 ml/dl)，将斑马鱼浸泡在交替葡萄糖溶液(营养液和 2%葡萄糖溶液)中(血糖水平升至 400 ml/dl 并持续较长时间)等[5]。以上方面结合斑马鱼在成像方面的巨大优势、再生能力和已建立的遗传方案，使其成为糖尿病晚期并发症的良好模型。

糖尿病性视网膜病变是导致糖尿病患者失明的原因之一，其主要特征是出现新生血管和血管渗漏。Alvarez [6]利用交替葡萄糖浓度处理成年斑马鱼 30 天建立斑马鱼糖尿病模型，发现斑马鱼视网膜尤其中央视网膜血管扩张增厚。另外，延长葡萄糖处理时间或者使用甘露醇处理会引起高渗作用，从而改变视网膜血管屏障，表现为更加广泛的紧密黏附粘连。除此之外，斑马鱼视网膜神经还发生了退行性性变以及视锥细胞功能障碍。Olsen 等[7]给成年斑马鱼注射去脲佐菌素诱导糖尿病模型，发现其肾小管基膜增厚，且由于细胞增殖能力减弱，受伤的尾鳍出现迟发性愈合[7]，该现象为斑马鱼用作糖尿病创伤愈合模型提供了基础。其他关于斑马鱼的研究主要集中在心脏发育和心脏标志物的表达上。据报道，斑马鱼体内升高的血糖浓度改变了心脏标志物的表达(tbx5, tbx20, has2)，并诱导心脏畸形，表现为心脏发育的循环缺陷，而心脏祖细胞和心脏管的未受影响[8]。据研究表明，斑马鱼体内高血糖的分子代谢机制以及抗糖

药物(甲福明二甲双胍、格列吡嗪、罗格列酮)治疗效果和哺乳动物相似以上研究结果结合斑马鱼的成像技术、高度复杂的遗传方法和重要治疗途径的独特机会有助于了解葡萄糖和葡萄糖代谢物诱导的并发症，明确病理生理机制，并发现新的治疗方法[9]。

3. 斑马鱼多发性骨髓瘤中研究

迄今为止，基因表达谱和其他基因组数据都未能准确预测多发性骨髓瘤患者个体的治疗效果，而较新的小鼠多发性骨髓瘤(multiple myeloma, MM)模型虽已有所发展，但它们预测临床反应的能力仍然有限[10] [11] [12] [13]。小鼠的肿瘤异种移植模型的营养材料(人类胎儿骨碎片)难获取、移植率低以及肿瘤生长滞后，这些限制突出了对一种新型模式生物的需求，这种生物能让人类 MM 细胞在体内的大规模快速繁殖并且可以用于治疗前药物反应预测和药物筛选。在这基础上，Jianhong [14]建立了一种新的斑马鱼模型，该模型支持 MM 细胞系和原代 MM 细胞的生长，并可以为药物筛选和多发性骨髓瘤研究生物学研究提供一个高效、快速和廉价的平台。

Jianhong 将 CM-Dil 标记的人 MM 细胞和前 138 + M 细胞通过显微注射移植至去绒毛化斑马鱼胚胎的卵黄周，24 h 后筛选造模成功的斑马鱼移入 24 孔无涂层板分组培育，通过荧光显微镜评估 24、48 和 72 hpt 的肿瘤生长和鱼的存活情况，测量显微照片上的荧光面积和密度，并估计对照组和药物治疗组动物的肿瘤体积。结果显示超过 80%的斑马鱼幼鱼体内多发性骨髓瘤细胞能存活九天，为药物筛选提供了足够的时间。接下来检测了 MM1s 细胞系、huIL-6 依赖的 INA-6 MM 细胞系和纯化的 CD138+原代人 MM 细胞在斑马鱼体内的生长，并观察到在不添加 huIL-6 或骨髓基质细胞的情况下它们的生长长达 96hpi，这表明斑马鱼胚胎的卵黄周围空间再现了人类骨髓微环境提供的生长支持。同时他们将多种抗 MM 药物(硼替佐米、来那度胺、地塞米松、AZD6244，17-AAG，AS703026 和雷帕霉素)对斑马鱼处理的结果与对患者处理结果相比较，观察到在斑马鱼模型中，来自硼替佐米耐受或来那度胺耐受患者的细胞也对同样的药物产生耐药性，验证了模型的可靠性，并证实了斑马鱼原发性 MM 异种移植模型可用于预测患者对体内化疗的反应。

这种斑马鱼胚胎异种移植模型能够使用病人细胞，所需的 MM 细胞数量少，减少动物之间的可变性，能够进行中通量体内药物筛选，和一个短的潜伏期可以允许实时快速筛选，支持 MM 细胞系和原代患者细胞的生长。可用于预测药物敏感性，并有可能用于临床前药物筛选。

4. 斑马鱼神经精神病研究

斑马鱼发育快、寿命较长、基因组特征良好且核酸序列已知[15]，与人类有高度的生理和遗传相似性，它们有相似的中枢神经系统形态 ，包括大脑的一般宏观组织和细胞形态[16] [17] [18] [19] [20]，例如，缰核是上丘脑中高度保守的结构，调节血清素和多巴胺的释放，与抑郁症的发生有关[21]，在患有抑郁症的啮齿动物模型[22]，以及在表现出抑郁相关应激行为的斑马鱼中过度激活[21]。说明了斑马鱼和哺乳动物的大脑底物之间的相似之处，以及斑马鱼的脑病研究对研究病理行为的翻译价值。斑马鱼拥有所有主要的神经介质系统，包括神经递质受体、转运体和合成和代谢酶，类似于在人类和啮齿动物中观察到的酶，因此被用于神经精神疾病研究[23] [24] [25]。

抑郁症是最广泛、令人严重衰弱的大脑疾病之一，全球 20%的人口在某个阶段都遭受到抑郁症的影响[26]。与抑郁症密切相关的各种遗传因素、环境刺激和神经化学紊乱似乎在斑马鱼的表型中也发挥了类似的作用。例如，糖皮质激素受体基因突变的 gr-s357 型斑马鱼表现出异常的皮质激素生物反馈、糖皮质激素水平升高和行为异常(运动能力减弱，习惯性障碍，过度惊吓)，与临床抑郁症患者的表型相似[27]。有趣的是，临床抗抑郁药(如选择性血清素再摄取抑制剂 SSRIs)使一些斑马鱼突变表型正常化，其效果与

在人体内相同，都改变了糖皮质激素信号并减轻应激障碍，这也证实了血清素能调节斑马鱼应激反应[27] [28]。除了基因遗传外，其他因素如慢性刺激源，通常会在临床和动物研究中触发情感性发病机制[29]。近期研究已经成功地将慢性不可预测压力(CUS)应用于斑马鱼，影响了斑马鱼浅滩、探索和焦虑行为，并改变脑蛋白质组谱和神经形成(啮齿动物模型中情感障碍的标志)；同时还出现出慢性刺激诱导的记忆缺陷和皮质醇水平升高[30] [31]，与人类和啮齿动物的抑郁状态相似。为了补充遗传和实验操作，药理学模型也被广泛应用于大脑研究。利血平(一种囊泡性单胺转运体的阻断剂)处理和相关的神经化学物质以及行为缺陷通常被用作啮齿动物的抑郁症模型，但也可以唤起斑马鱼的抑郁样行为，包括运动减退和浅滩行为中断，类似于临床抑郁症中观察到的运动迟缓和社会戒断症状，该药物处理斑马鱼的效果强调了单胺失调对抑郁症的影响，也为斑马鱼在模拟复杂的情感性脑障碍方面的发展效用提供依据[32]。

自闭症代表另一类严重的行为障碍，有着高遗传可能性(>90%)，影响了全球总人口的 1%~2% [33] [34]。一些证据表明斑马鱼能被用于自闭症研究。首先，有关自闭症的社会缺陷相关的各种模型(如社会互动、社会偏好)已从啮齿动物研究中改编，并成功应用于斑马鱼模型[35]。其次，由于为斑马鱼开发了优秀的遗传工具，该物种有望成为一种包括 ASD 在内的有用的高遗传性人类疾病的模式生物。例如，人类的 16p11.2 基因位点与 ASD 密切相关，而斑马鱼的同源区域涵盖了对大脑发育很重要的基因[36]。同样，编码肝细胞生长因子/分散因子(HGF/SF)的跨膜受体酪氨酸激酶的 MET 基因突变，会提高患自闭症风险[37] [38]。值得注意的是，斑马鱼基因敲除会损害小脑的发育和面部运动神经元的迁移[39]。由于这些基因对斑马鱼的大脑发育很重要，而 ASD 被认为是一种神经发育障碍，这些发现可能与 ASD 的发病机制有关，并表明斑马鱼模型具有很强的翻译相关性[40] [41]。斑马鱼的胚胎发育已经被彻底描述，因为这些鱼在生命的前 2 周实际上是透明的，在此期间大多数发育发生变化。因此，由于 40 多年对斑马鱼的发育生物学研究，该物种已成为胚胎学家最强大的脊椎动物工具之一，因此可能为与 ASD20 相关的发育异常的难题提供独特有效的答案。

药物致病是生物医学研究的一个重要领域。其中一个关键的例子是成瘾，一种常由药物滥用引起的普遍性疾病。除传统的啮齿动物模型之外，斑马鱼是研究奖励和药物滥用的有效翻译模型。幼鱼和成年斑马鱼对各种药物滥用、耐受性、明显的偏好(奖赏刺激)和戒断症状表现出高敏感性[42] [43]。例如，酒精对斑马鱼的影响研究，揭示了鱼类的许多行为变化，与啮齿动物和人类的变化相似[44] [45] [46] [47]。急性酒精在低剂量时会减少斑马鱼的恐惧/焦虑(增加“顶部”居住或减少不稳定的运动，也表现出刺激作用，增加斑马鱼的速度和攻击性)，而高剂量则会导致嗜睡和镇静[44]。这些变化显示了斑马鱼的双相轨迹，与啮齿动物和人类高度相似。长期接触酒精斑马鱼表现和哺乳动物相似[48]。在持续接触酒精后，当受到高急性剂量的短暂刺激时，斑马鱼对这种刺激的行为反应明显减少(或缺失)。同样，长期酒精暴露的戒断会导致一些行为和生理异常，类似于人类或啮齿动物的焦虑性戒断症状[49]。

5. 未来发展方向

斑马鱼模型被广泛应用于心血管疾病研究，斑马鱼胚胎心脏在 3wpf 时与人类胚胎心脏相似。它分为心房和心室，内附有心内膜，并且心房和心室之间会形成心脏瓣膜。为我们深入了解心脏发育提供了独特条件。斑马鱼具有体外受精、高繁殖能力、快速发育和易饲养等优势，使得它们在遗传学研究中特别易于操作。通过基因操作和荧光标记等方法，可用来观察了解心脏发育的形态学变化和调控机制，为未来治疗研究提供理论基础[50]。

神经退行性疾病是一类因某些因素导致神经元损失和功能丧失的疾病，如阿尔茨海默病和帕金森病等。研究人员可以通过基因操纵技术、物理刺激和药物处理运用斑马鱼模拟神经退行性疾病的特征，并观察分析相关的病理过程深入了解神经元死亡、蛋白质聚集和氧化应激等相关病理过程[51]。

斑马鱼的血糖调节机制以及对糖尿病治疗药物的反应与人体相似，如胰岛素 a 的介导使血糖降低，将来可用于糖尿病发病机制、治疗药物筛选和治疗效果研究[52]。

斑马鱼模型在研究肿瘤发生和发展的机制方面具有独特优势。通过基因编辑技术和注射肿瘤异种移植物的方法，研究人员可以模拟肿瘤的发生，并观察肿瘤形态学特征和生长模式。此外，斑马鱼模型还可用于研究肿瘤细胞的迁移、侵袭和转移等重要过程，为我们理解肿瘤转移机制提供新的视角。斑马鱼模型在肿瘤药物筛选和治疗研究中也具有重要的应用价值。

由于斑马鱼的生长速度快，数量较多，并且荧光标记技术的应用，使得研究人员能够高通量地筛选和评估化合物的抗肿瘤活性。斑马鱼模型还被用于研究肿瘤的靶向治疗策略，探索新的治疗靶点和药物递送系统。这些研究有望为肿瘤治疗提供新的思路和方法。通过斑马鱼模型在肿瘤发生和发展研究以及肿瘤药物筛选和治疗研究中的应用，我们可以更全面地理解肿瘤的发生机制，并探索创新的治疗策略，为临床肿瘤治疗提供新的突破。

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