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成年斑马鱼脊髓损伤修复中脑*gdnf*和*nos*基因的表达

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摘要 成年斑马鱼(*Danio rerio*)具有很强的脊髓损伤后自主修复的能力, 但目前其机制不明。为了研究斑马鱼中脑组织对脊髓再生的影响, 文章应用成年斑马鱼脊髓损伤模型, 采用实时定量PCR方法和原位杂交技术, 检测了斑马鱼脑中胶质细胞源性神经营养因子(*gdnf*)和一氧化氮合酶(*nos*)基因在脊髓损伤后4 h、12 h、6 d、11 d的表达情况, 展示了这两种基因在斑马鱼脑内不同核团的动态表达变化。结果显示, 成年斑马鱼脊髓损伤后, 神经营养因子*gdnf*基因在损伤急性期(4 h、12 h)和神经修复期(6 d、11 d)于斑马鱼脑内呈现显著性升高($P<0.05$),而一氧化氮合酶基因nos的表达于损伤急性期显著性升高 ($P<0.05$), 随后下降, 并在修复期 (11 d)显著降低($P<0.05$)。这表明, 脊髓损伤后, 高表达*gdnf*基因同时低表达*nos*基因的脑环境给脊髓损伤提供了良好的神经再生微环境, 从而可能促进轴突的再生长及运动能力的恢复。

关键词: 脊髓损伤 神经修复 斑马鱼 *gdnf* *nos*

Abstract: Recently, it is unclear about the mechanism of notable regenerated ability of adult zebrafish after spinal cord injury. To investigate the effects of brain on restoration from spinal cord injury, adult zebrafish spinal cord injury model was built and brain samples were dissected at different time points after the injury. Real-time quantitative PCR and *in situ* hy-bridization were applied to reveal the dynamics of glial cell line-derived neurotrophic factor (*gdnf*) and nitric oxide synthases (*nos*) mRNA expression in various regions of zebrafish brain. The results showed that, compared to sham group at each time points separately, the expression of *gdnf* mRNA in adult zebrafish brain during both acute phase (4 h and 12 h) and chronic phase of neuroregeneration (6 d and 11 d) increased significantly ($P<0.05$). The expression of *nos* mRNA in zebrafish brain enhanced during acute phase, and then reduced to the level lower than the sham group during the chronic phase of neuroregeneration (11 d) ($P<0.05$). This suggests that brain may promote neural axons regeneration in spinal cord via a more beneficial microenvironment which retains higher level of *gdnf* and lower level of *nos*.

Keywords: spinal cord injury, neural regeneration, zebrafish, *gdnf*, *nos*

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[1] Fang P, Lin JF, Pan HC, Shen YQ, Schachner M. A surgery protocol for adult zebrafish spinal cord injury. *J Genet Genomics*, 2012, 39(9): 481-487.

[2] Kizil C, Kaslin J, Kroehne V, Brand M. Adult neurogenesis and brain regeneration in zebrafish. *Dev Neurobiol*, 2012, 72(3): 429-461.

[3] Becker T, Bernhardt RR, Reinhard E, Wullimann MF, Tongiorgi E, Schachner M. Readiness of zebrafish brain neurons to regenerate a spinal axon correlates with differential expression of specific cell recognition molecules. *J Neurosci*, 1998, 18(15): 5789-5803.

[4] Lin JF, Pan HC, Ma LP, Shen YQ, Schachner M. The cell neural adhesion molecule contactin-2 (TAG-1) is benifecial for functional recovery after spinal cord injury in adult zebrafish. *PLoS One*, 2012, 7(12): e52376.

[5] Mocchetti I, Wrathall JR. Neurotrophic factors in central nervous system trauma. *J Neurotrauma*, 1995, 12(5): 853-870.

[6] Chu TH, Wang L, Guo A, Chan VWK, Wong CWM, Wu W. GDNF-treated acellular nerve graft promotes motoneuron axon regeneration after implantation into cervical root avulsed-spinal cord. *Neuropathol Appl Neurobiol*, 2012, 38(7): 681-695.

[7] Reid AJ, Sun M, Wiberg M, Downes S, Terenghi G, Kingham PJ. Nerve repair with adipose-derived stem cells protects dorsal root ganglia neurons from apoptosis. *Neuroscience*, 2011, 199: 515-522.

[8] Lee MY, Chen L, Toborek M. Nicotine attenuates iNOS expression and contributes to neuroprotection in a compressive model of spinal cord injury. *J Neurosci Res*, 2009, 87(4): 937-947.

[9] Dayan K, Keser A, Konyalioglu S, Erturk M, Aydin F, Sengul G, Dagci T. The effect of hyperbaric oxygen on neuroregeneration following acute thoracic spinal cord injury. *Life Sci*, 2012, 90(9-10): 360-364.

[10] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCT Method. *Methods*, 2001, 25(4): 402-408.

[11] Pan HC, Lin JF, Ma LP, Shen YQ, Schachner M. Major vault protein promotes locomotor recovery and regeneration after spinal cord injury in adult zebrafish. *Eur J Neurosci*, 2013, 37(2): 203-211.

[12] Facello B, Castaldo L, De Martino L, Lucini C. Glial cell line-derived neurotrophic factor in Purkinje cells of adult zebrafish: an autocrine mode of action? *Neurosci Lett*, 2009, 465(2): 133-137.

[13] Tolbert DL, Clark BR. GDNF and IGF-I trophic factors delay hereditary Purkinje cell degeneration and the progression of gait ataxia. *Exp Neurol*, 2003,183(1): 205-219.

[14] Koo H, Choi BH. Expression of glial cell line-derived neurotrophic factor (GDNF) in the developing human fetal brain. *Int J Dev Neurosci*, 2001,19(6): 549-558.

[15] Jones J, Jaramillo-Merchán J, Bueno C, Pastor D, Viso-León M, Martínez S. Mesenchymal stem cells rescue Purkinje cells and improve motor functions in a mouse model of cerebellar ataxia. *Neurobiol Dis*, 2010, 40(2): 415-423.

[16] Kawakami H, Nitta A, Matsuyama Y, Kamiya M, Satake K, Sato K, Kondou K, Iwata H, Furukawa S. Increase in neurotrophin-3 expression followed by Purkinje cell degeneration in the adult rat cerebellum after spinal cord transection. *J Neurosci Res*, 2000,62(5): 668-674. 3.0.CO;2-N target="_blank">

[17] Wang TY, Morgan JI. The Purkinje cell degeneration (*pcd*) mouse: an unexpected molecular link between neuronal degeneration and regeneration. *Brain Res*, 2007, 1140: 26-40.

[18] Kimura S, Hosaka N, Yuge I, Yamazaki A, Suda K, Taneichi H, Denda H, Endo N. Cerebrospinal fluid concentrations of nitric oxide metabolites in spinal cord injury. *Spine* (Phila Pa 1976), 2009, 34(18): E645-E652.

[19] Hervera A, Negrete R, Leánez S, Martín-Campos JM, Pol O. The spinal cord expression of neuronal and inducible nitric oxide synthases and their contribution in the maintenance of neuropathic pain in mice. *PLoS One*, 2010, 5(12): e14321.

[1] 顾爱华 严丽锋.斑马鱼在再生医学研究中的应用及进展[J]. 遗传, 2013,35(7): 856-866

[2] 彭夕洋 陈婷芳 黄婷 江志钢 吴秀山 邓云.心脏特异表达绿色荧光斑马鱼模型的建立与评估[J]. 遗传, 2013,35(4): 511-518

[3] 孙永华.第一届全国斑马鱼PI大会在武汉召开[J]. 遗传, 2013,35(4): 549-0

[4] 李方方 李文庆 荆清.G蛋白偶联受体在血管发育中的作用[J]. 遗传, 2013,35(4): 459-467

[5] 王学耕 朱作言 孙永华 赵珏.鱼类核移植与重编程[J]. 遗传, 2013,35(4): 433-440

[6] 佟静媛，柳星峰，贾顺姬.Rbb4l促进TGF-β/Nodal信号转导和斑马鱼胚胎的背部发育[J]. 遗传, 2013,35(4): 477-487

[7] 刘新星 张雨田 张博.构建斑马鱼心脏损伤-再生模型的手术方法[J]. 遗传, 2013,35(4): 529-532

[8] 张春霞 刘峰.斑马鱼高分辨率整胚原位杂交实验方法与流程[J]. 遗传, 2013,35(4): 522-528

[9] 孙婷 谢翔 张剑卿 包静 汤川政 雷道希 邱菊辉 王贵学.水平回转培养对斑马鱼血管发育的影响[J]. 遗传, 2013,35(4): 502-510

[10] 李小泉，杜久林.幼年斑马鱼的视觉系统与捕食行为[J]. 遗传, 2013,35(4): 468-476

[11] 李辉辉 黄萍 董巍 朱作言 刘东.斑马鱼研究走向生物医学[J]. 遗传, 2013,35(4): 410-420

[12] 沈延 黄鹏 张博.TALEN构建与斑马鱼基因组定点突变的实验方法与流程[J]. 遗传, 2013,35(4): 533-544

[13] 徐冉冉 张从伟 曹羽 王强.缺失*mir122*抑制斑马鱼肝脏前体细胞向肝细胞分化[J]. 遗传, 2013,35(4): 488-494

[14] 李礼，罗凌飞.以斑马鱼为模式动物研究器官的发育与再生[J]. 遗传, 2013,35(4): 421-432

[15] 黄玉斌，邹苏琪，殷梧，王昆，王晗，胡兵.成年斑马鱼OKR行为学分析[J]. 遗传, 2012,34(9): 1193-1201