Egr1 gene knockdown affects embryonic ocular development in

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摘要

Abstract

PURPOSE: To identify the changes in zebrafish embryonic ocular development after early growth response factor 1 (Egr1) gene knockdown by Egr1-specific translation inhibitor, morpholino oligonucleotides (MO). METHODS: Two kinds of Egr1-MO were microinjected separately with various dosages into one to four celled zebrafish embryos to find an optimal dose generating an acceptable mortality rate and high frequency of specific phenotype. Chordin-MO served as the positive control; a 5 mismatch MO of Egr1-MO1 and a nonspecific MO served as negative controls. We graded the Egr1 morphants according to their gross abnormalities, and measured their ocular dimensions accordingly. Western blot analysis and synthetic Egr1 mRNA rescue experiments confirmed whether the deformities were caused by Egr1 gene knockdown. Histological examination and three kinds of immunohistochemical staining were applied to identify glutamate receptor one expression in retinal ganglion cells and amacrine cells, to recognize acetylated alpha-tubulin expression which indicated axonogenesis, and to label photoreceptor cells with zpr-1 antibody. RESULTS: After microinjection of 8 ng Egr1-MO1 or 2 ng Egr1-MO2, 81.8% and 97.3% of larvae at 72 h postfertilization had specific defects, respectively. The gross phenotype included string-like heart, flat head, and deformed tail. The more severely deformed larvae had smaller eyes and pupils. Co-injection of 8 ng Egr1-MO1 and supplementary 12 pg synthetic Egr1 mRNA reduced the gross abnormality rate from 84.4% to 29.7%, and decreased the severity of deformities. Egr1 protein appeared in the wildtype and rescued morphants, but was lacking in the Egr1 morphants with specific deformities. Lenses of Egr1 morphants were smaller and had some residual nucleated lens fiber cells. Morphants' retinal cells arranged disorderly and compactly with thin plexiform layers. Immunohistochemical studies showed that morphants had a markedly decreased number of mature retinal ganglion cells, amacrine cells, and photoreceptor cells. Retinal axonogenesis was prominently reduced in morphants.

CONCLUSIONS: The Egr1 gene plays an important role in zebrafish embryonic oculogenesis. Ocular structures including lens and retina were primitive and lacked appropriate differentiation. Such arrested retinal and lenticular development in Egr1 morphants resulted in microphthalmos.