Production of the Novel Anti-hypertensive Peptide by Oocytes of Zebrafish(Danio rerio)

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ABSTRACT

In Taiwan, hypertension is one of the ten leading causes of death and is the major controllable risk factor associated with cardiovascular disease. Angiotensin converting enzyme (ACE) inhibitory peptides have attracted particular attention and have been studied widely for their applications to prevent hypertension among the bioactive peptides derived from milk proteins. A novel anti-hypertensive peptide (AP1), which derived from milk protein fermented by a nature symbiotic microbial starter-kefir and islated by Dr. Chen at National Chung-Hsing University and Dr. Chen at Da-Yeh University, has a significant effect on reducing systolic and diastolic blood pressure in spontaneously hypertensive rat. Though AP1 can be produced by E coli. and yeast, its post-translational modification and bioactivity is still not clear. The AP1 fragment (202 bp) was constructed with the promoters of vitellogenin, ovarian tumour or female-specific zebrafish zona pellucida genes into pAAV-IRES-VTG/OTU/ZPC-hrGFP and pEGFP-N1 vectors, and these vectors were transfected into tilapia ovarian (TO-2) cells or microinjected into the zebrafish oocytes to establish the transgenic fish line. RT-PCR or western blot analysis revealed that green fluorescent protein (GFP, 55 kDa) and AP1 peptide (8 kDa) could be expressed, observed, and detected after transfection or microinjection. Eggs produced by the transgenic fish showed green fluorescence and lasted to 2 months post fertilization. Only 6 eggs of 96 eggs (6.2%) from founder showed green fluorescence, and whose bioactivity would be further studied. The application of the platform techniques can be an alternative for the development of blood pressure controlling health food or even pharmaceuticals, and can be patented for commercial purpose or for the purpose of biosafety and biomedical researches.

Keywords : hypertension、anti-hypertensive peptide、tilapia ovarian cells、zebrafish、oocytes

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