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Studies on Effects of Ea4-Peptide of Rainbow Trout Pro-IGF-I on Heart Development, Vasculogenesis and Hematopoiesis in Fish Embryos by Transgenesis

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Insulin-like growth factors (IGFs) are mitogenic peptide hormones that regulate embryonic development, postnatal growth and cellular differentiation in vertebrates. IGFs are initially translated as pre-pro-peptides and then proteolytically processed to yield the mature IGFs and E-peptides. Like the C-peptide of pro-insulin, the E-peptides of pro-IGFs are generally believed to possess little or no biological activity other than their potential roles in the biosynthesis of the mature IGFs. Like human IGF-I, previous studies in our laboratory showed that the recombinant trout Ea4-peptide of pro-IGF-I exhibited a dose-dependent mitogenic activity in cultured BALB/3T3 fibroblasts and other non-oncogenic transformed cells (Tian *et al.*, 1999). We have also shown by *in vitro* and *in vivo* studies that Ea4-peptide possessed novel anti-tumor activities (Chen *et al.*, 2002; Kuo and Chen, 2002; Kuo and Chen 2003). Recent results of studies conducted in chorionicallantoic membrane of developing chicken embryos revealed that Ea4-peptide of trout pro-IGF-I also possesses a dose-dependent antiangiogenic activity. Together these results raised the question whether Ea4-peptide of trout pro-IGF-I may affect heart and blood vessel development and hematopoiesis in fish embryos.

To answer this question, a transgene construct expressing secreted Ea4-peptide of trout pro-IGF-I was constructed and introduced into one-cell stage medaka embryos by electroporation. Three different phenocopies of heart developmental defects were observed in many embryos: (i) embryos arrested at the cardiomyocyte stage; (ii) embryos arrested at

the heart tube stage; and (iii) embryos arrested at the heart tube looping stage. Results of PCR analysis of genomic DNA and RT-PCR analysis of total RNA from these defective embryos showed the presence as well as the expression of Ea4-peptide transgene. Although the overall development of embryos arrested in heart tube looping stage can proceed to post-hatching, the hatched animalsare non-motile and die in a few days after hatching. Obvious defects in vasculogenesis and hematopoiesis are also observed in all defective embryos. These results clearly show that the presence of Ea4-peptide of trout pro-IGF-I can arrest heart and blood vessel development and hematpoiesis in fish embryos. Research is underway to identify genes in heart development, vasculogenesis and hematopoiesis pathways that are regulated by the Ea4-peptide of trout pro-IGF in fish. (Research results presented in this paper are supported by grants from NFS and USDA to TTC)