Gene Expression of Insulin-like Growth Factor-I Receptor and p53 Suppressor during Zebrafish (Danio rerio) Embryogenesis

Ronshan Cheng¹ and Jen-Leih Wu²,*

¹Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan, Republic of China
²Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

Abstract: In vertebrates insulin-like growth factors (IGFs) regulate important cellular activities involving proliferation, differentiation, and antiapoptosis and their biological activities are mediated through the insulin-like growth factor-I receptor (IGF-IR). To understand the functions of IGF-IR in zebrafish embryogenesis, the polymerase chain reaction (PCR) cloning technique was applied to isolate the IGF-IR gene. A 5'-truncated 3285-nucleotide zebrafish IGF-IR sequence was assembled from 3 overlapping clones. This contained a partial coding region of 1550 nucleotides and a 1735-nucleotide 3' untranslated region. The deduced 515 amino acid residues included the conserved kinase domain and shared 60.9%, 61.1%, and 59.9% homology to human, mouse, and frog, respectively. To understand the relationship of IGF-IR with p53 suppressor gene during embryogenesis, expression of both genes was analyzed in parallel by semicompetitive reverse transcriptase PCR and whole-mount in situ hybridization. This analysis indicated that messenger RNA of both genes was of maternal origin, but the p53 suppressor mRNA was relatively more abundant than the IGF-IR message in most of the developmental stages, except possibly at 28 hours postfertilization. At this stage the IGF-1 receptor message was highly expressed and visible in whole internal organ regions by whole-mount in situ hybridization, while p53 message was concentrated in the head portion and barely detectable in the trunk portion. The results suggest that IGF-IR and p53 mRNA are expressed at different places and different times. However, the temporal and spatial relationship of IGF-IR and its relationship to p53 suppressor protein during developmental processes remain unknown.

Key words: insulin-like growth factor-I receptor, p53 tumor suppressor, embryonic, zebrafish.

INTRODUCTION

In vertebrates it is generally recognized that growth hormone exerts its effects through insulin-like growth factor-I (IGF-I) to induce downstream signals important in embryonic and postnatal development (Bischell et al., 1992). In fish, like other vertebrates, IGF-I is a ubiquitous peptide that circulates at high levels in serum and is expressed in multiples tissues including early embryo and unfertilized eggs (Moriyama et al., 1995; Greene and Chen, 1997; Chen et al., 2001). It has a broad spectrum of effects, including stimulation of embryonic and postnatal growth, various metabolic effects, participation in tissue regeneration, and
cellular activities such as transformation, proliferation, differentiation, and antiapoptosis (reviewed in Rosenthal, 1999; Zapf et al., 1999; Baserga, 2000).

The effects of IGF-I are mediated via binding to a specific heterotetrameric membrane receptor, the IGF-I receptor (IGF-IR), which consists of 2 extracellular α-subunits and 2 membrane-spanning β-subunits with tyrosine kinase activity. The major biological function of IGF-IR is to transmit ligand-binding stimuli from extracellular to intracellular spaces by phosphorylating downstream substrates, which then initiate a series of signal transductions from cytoplasm to nucleus (reviewed in Werner, 1999). The number of IGF-IR per cell has been implicated in the modulation of mitogenic, transforming, and antiapoptotic activities within cells (Rubini et al., 1997; Baserga et al., 1999). Overexpression of IGF-IR is considered to be one of the dominant mechanisms of tumorigenesis; however, down-regulation of IGF-IR levels can reverse the transformed phenotype and render the cells more sensitive to apoptosis in vivo.

Because of its importance at the physiological level, the regulation of IGF-IR gene expression has been thoroughly investigated (Werner et al., 1993, 1996; Hernandez-Sanchez et al., 1997; Chambery et al., 1999; Damon et al., 2001). Among the regulators, p53 protein, well known for its tumor suppressor and cell-cycle checkpoint control function, is an important regulator of IGF-IR that represses its expression and is also implicated as a vital protein in embryonic development (reviewed in Levine, 1997; Hall and Lane, 1997; Choi and Donehower, 1999; Prives and Hall, 1999; Hara et al., 2000; Lohrum and Vousden, 2000; Hikasa and Taira, 2001). The p53 suppressor protein can also transactivate the insulin-like growth factor binding protein-3 (IGFBP-3), which inhibits IGF-I biological activities by forming a binary complex and prevents its association with IGF-IR (Buckbinder et al., 1995).

The zebrafish (Danio rerio), with the advantages of short generation time, transparent embryos, and ease of breeding and maintenance, has received considerable attention as a model for vertebrate embryology and developmental biology (Weinberg, 1992). Despite their pleiotropic effects in postnatal growth, the exact roles of IGF-IR and p53 suppressor genes in embryogenesis are largely unknown. In this study we have cloned a partial complementary DNA sequence of IGF-IR from zebrafish. The sequence includes an open reading frame of 1550 nucleotides and 1735 nucleotides of 3' untranslated region. The deduced amino acid sequence shares approximately 60% identity with that of vertebrate counterparts.

A preliminary experiment to study the early relationship of p53 and IGF-IR genes in zebrafish embryogenesis was carried by semicompetitive reverse transcriptase polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization. Both RT-PCR and whole-mount in situ hybridization indicated that the IGF-IR message was maternally supplied and expressed throughout embryonic development. The semicompetitive RT-PCR indicated that p53 messenger RNA was more abundant than IGF-IR in most developmental stages. Around 28 hours postfertilization (hpf), IGF-IR gene expression increased sharply. Whole-mount in situ hybridization also indicated that at 28 hpf the IGF-IR was highly expressed during trunk formation, while p53 suppressor gene expression was barely detectable in this region. This supports the conclusion that cellular proliferation is a counterbalanced effect of p53 and IGF-IR during embryogenesis.

**Materials and Methods**

**Animals and DNA Reagents**

The zebrafish (Danio rerio) AB strain was fed twice daily and maintained at 28°C with 12-hour photoperiods at our aquarium facility. The egg collection and developmental stage classification followed the Zebrafish Book (Westerfield 1998). All the PCR primers used in this experiment (Table 1) were synthesized by local agents (MDBio, Inc., Taiwan), and DNA sequencing was performed on an ABI Model 377 automated DNA sequencer at Mission Biotech, Inc., Taiwan.

**Preparation of Zebrafish cDNA Template**

The 1-month-old whole zebrafish total RNA was isolated by Trizol reagent following the manufacturer’s protocol (Gibco BRL, Rockville, Md.). The cDNA was prepared from 5 μg of total RNA by Expand reverse transcriptase (Boehringer Mannheim, Mannheim, Germany) following the manufacturer’s protocol. The cDNA was synthesized by our own anchor primer, the D(T) in Table 1, which permits the use of R(T) adapter primer (Table 1) in 3'-rapid amplification of cDNA ends (RACE; Frohman et al., 1988).