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Distinct organ-specific up- and down-regulation of IGF-I and IGF-II mRNA in various organs of a GH-overexpressing transgenic Nile tilapia

Elisabeth Eppler · Giorgi Berishvili · Peter Mazel · Antje Caelers · Gyulin Hwang · Norman Maclean · Manfred Reinecke

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Abstract Several lines of GH-overexpressing fish have been produced and characterized concerning organ integrity, growth, fertility and health but few and contradictory data are available on IGF-I that mediates most effects of GH. Furthermore, nothing is known on IGF-II. Thus, the expression of both IGFs in liver and various extrahepatic sites of adult transgenic (GH-overexpressing) tilapia and age-matched wild-type fish was determined by real-time PCR. Both IGF-I and IGF-II mRNA were found in all organs investigated and were increased in gills, kidney, intestine, heart, testes, skeletal muscle and brain of the transgenics (IGF-I: 1.4–4-fold; IGF-II: 1.7–4.2-fold). Except for liver, brain and testis the increase in IGF-I mRNA was higher than that in IGF-II mRNA. In pituitary, no significant change in IGF-I or IGF-II mRNA was detected. In spleen, however, IGF-I and IGF-II mRNA were both decreased in the transgenics, IGF-I mRNA even by the 19-fold. In agreement, in situ hybridisation revealed a largely reduced number of IGF-I mRNA-containing leukocytes and macrophages when compared to wild-type. These observations may contribute to better understanding the reported impaired health of GH-transgenic fish. Growth enhancement of the transgenics may be due to the increased expression of both IGF-I and IGF-II in extrahepatic sites. It is also reasonable that the markedly enhanced expression of liver IGF-II mRNA that may mimick an early developmental stage is a further reason for increased growth.

Keywords Growth hormone · IGF-I · IGF-II · Transgenic · Liver · Muscle · Gills · Kidney · Intestine · Heart · Spleen · Brain · Pituitary

E. Eppler · G. Berishvili · P. Mazel
Research Group Neuro-Endocrine-Immune Interactions, Institute of Anatomy, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

A. Caelers · M. Reinecke (✉)
Division of Neuroendocrinology, Institute of Anatomy, University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland
e-mail: reinecke@anatom.uzh.ch

G. Hwang · N. Maclean
Division of Cell Science, School of Biological Sciences, University of Southampton, Hampshire SO16 7PX, UK

Introduction

The insulin-like growth factors (IGF-I, IGF-II) belong to a family of hormones with structural similarities to insulin. Whereas IGF-I is known as potent mitogenic hormone that induces growth and differentiation in a variety of target organs (Reinecke and Collet 1998), the role of IGF-II is still enigmatic (Reinecke et al. 2005). Most studies on the IGF-system in non-mammalian vertebrates deal with bony fish which are of special commercial value. Thus, scientific results on growth regulation in fishes

are not only relevant for basic research but also for aquaculture.

In mammals, IGF-I is mainly produced in liver, the principal source of endocrine IGF-I, whereby the primary stimulus for synthesis and release of liver IGF-I is growth hormone (GH) from the anterior pituitary (Reinecke and Collet 1998; Reinecke et al. 2005). In bony fish, the major site of IGF-I gene expression also is liver (Duan 1998; Reinecke et al. 2005; Wood et al. 2005) and GH injections promoted liver IGF-I mRNA expression and increased IGF-I serum levels in salmonids, seabream and tilapia (Reinecke et al. 2005). In vitro, GH also stimulated IGF-I expression in primary hepatocyte cultures of salmonids (Shamblott et al. 1995; Pierce et al. 2005), tilapia (Schmid et al. 2000, 2003), and seabream (Leung et al. 2008). Furthermore, there is evidence for extrahepatic IGF-I production in fishes (for references see: Caelers et al. 2004; Eppler et al. 2007a), especially during development (Berishvili et al. 2006a, b; Patruno et al. 2008) and for partial regulation by GH in extrahepatic sites (Tse et al. 2002; Vong et al. 2003; Biga et al. 2004; Eppler et al. 2007a).

In several bony fish IGF-II gene expression was detected in all IGF-I mRNA-expressing organs (Shamblott et al. 1995; Duguay et al. 1996; Collet et al. 1997; Loffing-Cueni et al. 1999; Schmid et al. 1999; Caelers et al. 2004; Moriyama et al. 2008) which raises the question whether or not the expressions of IGF-I and IGF-II may be simultaneously regulated in different organs. So far, only few and inconsistent data on GH action towards IGF-II gene expression have been obtained in seabream, rainbow trout, Japanese eel and carp (Shamblott et al. 1995; Duguay et al. 1996; Perrot and Funkenstein 1999; Tse et al. 2002; Vong et al. 2003; Moriyama et al. 2008) and nothing is known for transgenic fish carrying exogenous GH gene constructs which provide valuable tools for the investigation of GH action on IGF-I and IGF-II expression. Furthermore, although investigations on transgenic fish have mainly dealt with growth parameters, fertility, body and organ integrity (Rahman et al. 1998; Maclean et al. 2002; Sundström et al. 2004), only few observations have been made on the immune system. Taking into account the relevance of farmed fish as nutrition factor, susceptibility to infection is important, especially since the enormous enlargement of aquaculture goes along with fish rearing at high density leading to increased infections.

There is evidence for neuroendocrine-immune interactions in fish (Segner et al. 2006; Bowden 2008) but interactions of the GH/IGF-I system and the immune system in fishes are not understood at all. So far, the GH receptor has been detected on hematopoetic cells and in head kidney of gilthead seabream (Calduch-Giner and Pérez-Sánchez 1999; Sitjà-Bobadilla et al. 2008), and tilapia (Shved et al. 2009), IGF-I gene or peptide expression in lymphoid tissues of several fish species including tilapia (for references see: Shved et al. 2009), and the IGF-I receptor on immune cells and in head kidney of gilthead seabream (Funkenstein et al. 1997; Sitjà-Bobadilla et al. 2008). Expression of both IGF-I and IGF-II genes was described in tilapia spleen (Caelers et al. 2004; Shved et al. 2009) and gilthead seabream and tilapia head kidney (Sitjà-Bobadilla et al. 2008; Shved et al. 2009).

Thus, only few and contradictory results are available on the expression of IGF-I in transgenic fish, and nothing is known on IGF-II. Thus, the aim of the present study was to investigate the long-term regulation of IGF-I and IGF-II by GH in teleost fish. As experimental model we have chosen a transgenic (GH-overexpressing) tilapia (*Oreochromis niloticus*).

Materials and methods

Production, maintenance, and discrimination of transgenic and wild-type fish

As experimental model we used transgenic, GH-overexpressing *Oreochromis niloticus* previously produced from crosses between a wild type female *Oreochromis niloticus* and a G1 transgenic male. This line of growth-enhanced tilapia (C86) carries a single copy of a chinook salmon (*Oncorhynchus tschawytscha*) GH gene spliced to an ocean pout (*Macrozoarces americanus*) antifreeze promoter (OP-AFPcsGH) co-ligated with a carp beta actin/lacZ reporter gene construct, integrated into the tilapia genome (Rahman et al. 1998). Tilapia were bred in tanks at 24–25°C under a 13/11 h light/dark cycle and fed with trout pellets 3 times a day to satiation as described previously (Rahman et al. 1998). In order to discriminate the transgenic or non-transgenic state

of the individuals, PCR was carried out on DNA from fin clips. An approximately 3 × 2 mm clip was taken from the caudal fin of each individual investigated and immediately frozen in liquid nitrogen. Prior to fin clipping, fish were tagged with transponders (Fish Culture Research Institute, FCRI growth trial) to allow identification of each fish after PCR analysis. Isolation and purification of DNA and subsequent PCR and Southern Blotting were performed as previously described (Rahman et al. 1998) by using primers to detect novel junction fragments between the exogenous GH gene and the reporter gene.

Tissue sampling

17 months old individuals of the C86 strain of *Oreochromis niloticus* ($n = 10$) and non-transgenic siblings ($n = 10$) were used. Pituitaries and small tissue samples of liver, intestine, heart, brain, gills, kidney, spleen, skeletal muscle and testis were rapidly excised and, for RNA preservation, immediately transferred into 1.5 ml of RNAlater™ (Ambion, Austin, TX). The samples were kept overnight at 4°C and later stored at −20°C until RNA isolation. For in situ hybridisation, specimens were fixed by immersion in Bouin's solution without acetic acid for 4 h at room temperature, dehydrated in ascending series of ethanol and routinely embedded in paraplast (58°C).

Real-time PCR

Tilapia IGF-I, IGF-II and ß-actin specific primers and probes were designed as already described (Caelers et al. 2004) based on the sequences of *Oreochromis mossambicus* IGF-I (Reinecke et al. 1997), IGF-II (Chen et al. 1998), and *O. niloticus* ß-actin (Hwang et al. 2003). Total RNA was extracted using TRIzol®Reagent (Invitrogen, Belgium) and treated with 1 U of RQ1 RNAse-free DNase (Catalys, Switzerland). Samples were subjected individually to real-time PCR as already described (Caelers et al. 2004). In brief, triplicates of 10 ng of total RNA were subjected in parallel to real-time PCR using one-step RT-PCR Mastermix (Applied Biosystems, Rotkreuz, Switzerland). Each 25 µl RT-PCR mixture contained 12.5 µl 2× Master Mix (AmpliTaqGold®DNA Polymerase, dNTPs with dUTP, Passive Reference 1 and optimized buffer components), 0.625 µl 40× RT enzyme mix (Multi-Scribe™Reverse Transcriptase and RNase inhibitor), 300 nM of each primer, 150 nM of fluorogenic TaqMan probe and 1 µl of 10 ng/µl diluted RNA. A reverse transcription step of 30 min at 48°C and a denaturation step of 10 min at 95°C were followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Reverse transcription and amplification were carried out in a single tube using an ABI PRISM™ 7700 Sequence Detection System (Applied Biosystems).

Generation of probes for in situ hybridisation

Probes for in situ hybridisation were prepared as already described (Schmid et al. 1999; Berishvili et al. 2006a, b). In brief, total RNA from tilapia liver was extracted with the Ultraspec Extraction Kit (ams, Lugano, Switzerland). For cDNA synthesis 5 µg RNA were annealed with 1 µM of a poly(dT) primer (5′-CCTGAATTCTAGAGCTCAT(dT17)-3′) for 3 min at 70°C. The RNA/primer mix was incubated for 1 h at 37°C with 15 mM dNTPs and 10 U AMV-RTase (Pharmacia, Switzerland) in 1× reaction buffer. One microliter cDNA was incubated with 1 µM of sense and antisense primers corresponding to the B- and E- domain, 200 µM dNTPs, and 1 U Taq-polymerase (Pharmacia) in 1× incubation buffer. The amplification program was optimised for a Stratagene RoboCycler Gradient 40: 1 cycle 10 min at 94°C, 1 min at 59°C, 2 min at 72°C; 30 cycles 1 min at 94°C, 1 min at 59°C and 2 min at 72°C followed by final extension of 5 min at 72°C. PCR fragments were separated on a 2% agarose gel and eluted by the Gel Extraction Kit QIAquick (Qiagen, Switzerland). Subsequently, the PCR products were cloned in a pCR-Script SK(+) cloning vector using a kit (Stratagene, Heidelberg, Germany). Plasmids containing the gene sequence fragments were sequenced (Microsynth, Switzerland) and the sequences compared to database. The plasmids containing the specific inserts of IGF-I (207 bp) were used as templates for the synthesis of digoxigenin (DIG)-labelled RNA probes. Linearisation was performed with *EcoRI* for T3- and *NotI* for T7-polymerase-driven transcription. One µg of linearised plasmid was transcribed in vitro in the presence of DIG-UTP from T3 and T7 promotors to obtain antisense and sense probes. Integrity of probes and efficiency of labelling were confirmed by dot blot and

gel electrophoresis including blotting and incubation with antibody.

In situ hybridisation

Four µm sections on Super Frost Plus slides (Menzel-Gläser, Germany) were dewaxed, rehydrated in descending series of ethanol, and postfixed with 4% PFA and 0.1% GA in 1× PBS. The following steps were carried out with DEPC-treated solutions in a humidified chamber: sections were digested with 0.02% proteinase K in 20 mM Tris–HCl/pH 7.4, 2 mM $CaCl_2$ for 10 min at 37°C and treated with 1.5% triethanolamine and 0.25% acid anhydride for 10 min at room temperature. Slides were incubated with 100 µl prehybridisation solution per section for 3 h at 54°C. Hybridisation was carried out overnight at 54°C with 50 µl of hybridisation buffer containing 200 ng of sense or antisense probes previously denaturated for 5 min at 85°C. Slides were washed for 15 min at room temperature in 2× SSC, and for 30 min at 54°C at descending concentrations of SSC. Sections were incubated with alkaline phosphatase-coupled anti-DIG antibody diluted 1:4000 in 1% blocking reagent in buffer P1 for 1 h at room temperature in the dark. After washing, sections were treated with buffer P3, 5 mM levamisole and NBT/BCIP stock solution. Colour development was performed overnight at room temperature and stopped by rinse in tap water for 15 min. Sections were mounted with glycergel. Microscopy and photography were performed with a Zeiss Axioscope using the Axiovision 3.1 software (Zeiss, Zürich, Switzerland). Specificity of the probes has been previously demonstrated for tilapia male and female gonads (Schmid et al. 1999; Berishvili et al. 2006a), liver (Schmid et al. 1999), brain (Shved et al. 2007), pituitary (Eppler et al. 2007b), and head kidney (Shved et al. 2009).

Statistical analysis

All experimental data for IGF-I and IGF-II are expressed as *n*-fold changes of gene expression in the GH-transgenic fish relative to the wild type control level set as 1. The comparative threshold cycle ($\Delta\Delta C_T$) method was used to calculate relative gene expression ratios as previously described (Shved et al. 2007, 2009). Efficiency tests for β-actin, IGF-I and IGF-II assays (Caelers et al. 2004) permitted the accurate use of the $\Delta\Delta C_T$ method. Data were normalized to β-actin as reference gene. Stability of β-actin gene expression between transgenic and control fish was assured. Statistical significance was calculated using Mann–Whitney rank sum test, with an exact *P* value. Statistical analyses were performed with GraphPad Prism© 4.

Results

IGF-I and IGF-II mRNA expression in liver

IGF-I mRNA was slightly (1.391-fold, $P < 0.0028$) elevated in transgenics as compared to the control fish whereas IGF-II mRNA expression was pronouncedly elevated (4.232-fold, $P < 0.0075$) (Table 1).

IGF-I and IGF-II mRNA expression in skeletal muscle

IGF-I mRNA was approximately doubled (1.996-fold, $P < 0.0075$) in transgenic skeletal muscle (Table 1) as compared to the control fish whereas IGF-II mRNA expression was less increased (1.655-fold, $P < 0.015$).

IGF-I and IGF-II mRNA expression in gills and kidney

In gills (Table 1), both IGF-I and IGF-II mRNA were approximately doubled (IGF-I: 2.050-fold, $P < 0.0066$; IGF-II: 2.022-fold, $P < 0.0075$) in the GH-transgenics as compared to the control fish. In kidney (Table 1), IGF-I gene expression (2.283-fold, $P < 0.0043$) was more pronouncedly enhanced than IGF-II mRNA expression (1.829-fold, $P < 0.0423$).

IGF-I and IGF-II mRNA expression in intestine

In intestine (Table 1), IGF-I gene expression was strongly elevated (4.019-fold, $P < 0.008$) in transgenics, whereas elevation of IGF-II mRNA was less pronounced but still twice (2.186-fold, $P < 0.025$) of that found in the control fish.

IGF-I and IGF-II mRNA expression in heart

IGF-I mRNA was markedly (2.283-fold, $P < 0.0062$) increased in the transgenics as compared to the

Table 1 Ratio (±standard deviation, SD) of IGF-I and IGF-II mRNA levels of transgenic GH-overexpressing tilapia organs as compared to wild-type (set as 1) gene expression levels

Organ	IGF-I			IGF-II			sGH[a]	
	Ratio	SD	*P*-value	Ratio	SD	*P*-value	pg/μg	SD
Liver	1.391	0.516	<0.0028	4.232	1.921	<0.0075	8.3	2.5
Muscle	1.996	0.817	<0.0075	1.655	0.833	<0.015	2.6	1.4
Gills	2.050	0.869	<0.0066	2.022	0.719	<0.0075	4.1	2.0
Kidney	2.283	0.426	<0.0043	1.829	0.741	<0.0423	0.2	0.08
Intestine	4.019	1.426	<0.008	2.186	0.997	<0.025	0.7	0.63
Heart	2.283	0.809	<0.0062	1.462	0.697	NS	1.9	0.8
Testis	2.287	1.251	<0.016	2.455	1.096	<0.011	2.0	1.7
Spleen	−18.804	3.033	=0.0013	−2.018	0.656	NS	0.6	0.52
Brain	1.353	0.449	<0.0001	2.038	0.379	<0.0025	1.4	0.5
Pituitary	1.192	0.180	NS	1.200	0.378	NS	ND	ND

NS not significant, *ND* not detected

[a] Data are presented together with absolute amounts (pg/μg total RNA) of the exogenous salmon (s)GH mRNA as previously published (Caelers et al. 2005)

controls while IGF-II mRNA expression was only slightly elevated (1.462-fold) in transgenics as compared to the control (Table 1).

IGF-I and IGF-II mRNA expression in testis

Both IGF-I and IGF-II mRNAs were strongly enhanced (IGF-I: 2.287-fold, $P < 0.016$; IGF-II: 2.455-fold, $P < 0.011$) in the GH-transgenics as compared to the control fish (Table 1).

IGF-I and IGF-II mRNA expression in spleen

In spleen, both IGF-I and IGF-II mRNAs were lower in the GH-transgenics as compared to the control fish (Table 1) whereby in the transgenic tilapia gene suppression was much more pronounced for IGF-I which was lowered by −18.804-fold ($P = 0.0013$) whereas IGF-II gene was suppressed by −2.018-fold.

The suppressed IGF-I gene expression in spleen was verified by in situ hybridisation (Fig. 1) where a similarly reduced IGF-I gene expression in leukocytes and macrophages was observed in the transgenics (Fig. 1b) when compared to the control fish (Fig. 1a). Moreover, in the transgenics, a larger area of the melanomacrophage centres was observed whereby the melanomacrophages were strongly reduced in number (Fig. 1b).

IGF-I and IGF-II mRNA expression in brain and pituitary

IGF-I mRNA was slightly (1.353-fold, $P < 0.0001$) elevated in brain of the transgenics as compared to

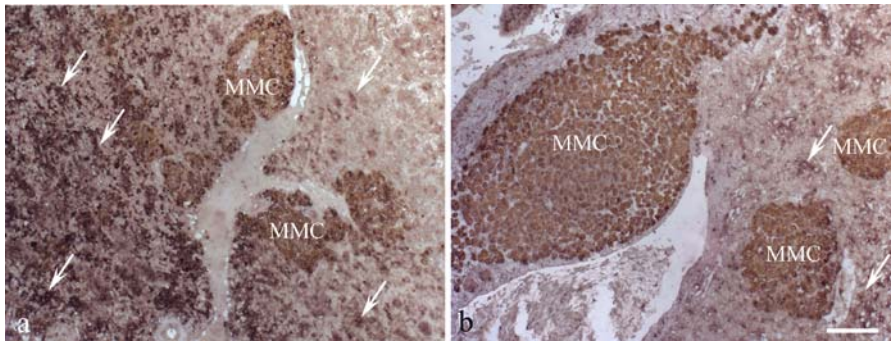

Fig. 1 In situ hybridisation of IGF-I gene expression in spleen and melanomacrophage centres (MMC) of **a** control and **b** transgenic fish. Enlarged MMC occur in the transgenic fish. In wild-type tilapia (**a**) much more leukocytes express IGF-I mRNA (*arrows*) than in the transgenic tilapia (**b**). *Bar*: 240 μm

the control fish. IGF-II mRNA expression was pronouncedly elevated (2.038-fold, $P < 0.0025$) in transgenics as compared to the control fish brain (Table 1). No significant effect (IGF-I: 1.192-fold; IGF-II: 1.200-fold) was observed in pituitary of the transgenic fish when compared to the wild-type fish (Table 1).

Discussion

In the present study, both IGF-I and IGF-II mRNA levels were increased in liver of the transgenics. For IGF-I this is consistent with previous reports in fish including our own transgenic tilapia (Eppler et al. 2007a) that GH treatment stimulates liver IGF-I gene expression. For IGF-II expression, no GH effect was found in gilthead seabream (Duguay et al. 1996) but, in accordance with our results, GH increased IGF-II mRNA levels in rainbow trout, common carp and Japanese eel (Shamblott et al. 1995; Vong et al. 2003; Moriyama et al. 2008), and in rainbow trout and redbanded seabream, similar to our findings in tilapia, more pronouncedly than IGF-I mRNA (Gahr et al. 2008; Ponce et al. 2008).

IGF-II mRNA in muscle was similarly increased in the GH-overexpressing fish as found previously for IGF-I mRNA (Eppler et al. 2007a) which suggests that expression of both IGFs in muscle is under control by GH. For IGF-I, this agrees with the work of Kajimura et al. (2001) who observed an increase in tilapia muscle IGF-I mRNA after GH injection and with results in transgenic GH-overexpressing coho salmon (Raven et al. 2008). Recently, it has been shown in muscle cells of rainbow trout that IGF-II exerts mitogenic and metabolic effects equivalent to those of IGF-I (Codina et al. 2008). Thus, the markedly enhanced growth of the transgenics (Rahman et al. 1998) probably is due to raised local expression of both, IGF-I and IGF-II in muscle.

In tilapia gills which possess the GH receptor (Fryer 1979) and express IGF-I at higher levels than IGF-II (Caelers et al. 2004), both IGFs were more intensely expressed in the transgenics. Together with similar results in GH-injected common carp (Vong et al. 2003) this strongly indicates that both IGFs in gills are under control by GH which is of particular relevance since GH and IGF-I are believed to play a major role in osmoregulation (e.g., Shepherd et al. 2005; Cao et al. 2009). Also in wild type tilapia kidney, IGF-I was expressed at a higher level than IGF-II (Caelers et al. 2004). In the GH-overexpressing tilapia, both IGF-I and IGF-II expressions were pronouncedly elevated although the GH-transgene was expressed at a relatively low level. This gives support to the hypothesis that the presence of the transgene is more important than its absolute amount (Hernández et al. 1997; Caelers et al. 2005). Similar to elevated IGF-I and IGF-II mRNA levels in common carp upon GH administration (Vong et al. 2003), in the present study in intestine of transgenic tilapia a four and twofold increase, respectively, in IGF-I and IGF-II expressions was found suggesting a role for both in the proposed enhanced food uptake and conversion of GH-transgenic fish (Devlin et al. 1995).

IGF-I and IGF-II mRNA expression were found in salmonid and tilapia testes and ovary (e. g. Shamblott and Chen 1993; Duguay et al. 1994; Schmid et al. 1999; Perrot et al. 2000; Biga et al. 2004) and absolute gene expression levels of both IGFs in wild type tilapia testis were second after liver (Caelers et al. 2004). In the present study, increased expressions of both IGFs were found in testis of the transgenic tilapia along with a considerable expression of the exogenous transgene (Caelers et al. 2005). Thus, our study suggests that both IGF-I and IGF-II act as local mediators of GH in fish testis, an assumption compatible with results in rainbow trout (Perrot and Funkenstein 1999) where GH and pituitary extracts induced eightfold and two to threefold increases in IGF-I and IGF-II mRNA levels, respectively, and with the presence of GH receptors in fish testis (Le Gac et al. 1992).

Surprisingly in spleen, IGF-I and IGF-II mRNA were in contrast to the other organs expressed at lower levels in GH-transgenic than control fish although GH is generally assumed to exert stimulatory effects on the immune system. For instance, GH increased unspecific leukocyte activity in rainbow trout, tilapia and gilthead seabream in vitro and in vivo (Kajita et al. 1992; Sakai et al. 1996, 1997; Calduch-Giner et al. 1997; Yada et al. 2002) and assisted in vitro the recovery of cortisol-treated rainbow trout leukocytes (Yada et al. 2004). Vice versa, hypophysectomy decreased superoxide anion production of tilapia head kidney leukocytes (Yada et al. 2002). In our study, life-long stimulation with

GH enlarged the melanomacrophage centres which are considered as primitive analogues of lymphnodes in higher teleosts (Agius and Roberts 2003). In the transgenics, they contained very few melanomacrophages which indicates a modified cell composition in the transgenic state. High mortalities of GH-transgenic coho salmon have been reported (Devlin et al. 1995) that may be caused by impaired immune function (Jhingan et al. 2003). For instance, in GH-transgenic amago salmon, lowered serum lysozyme activity was detected (Mori et al. 2007) in contrast to elevated lysozyme activity in GH-treated rainbow trout and channel catfish (Yada et al. 2001; Peterson et al. 2007) suggesting a modification of the innate immune system by the GH-transgenic state (Mori et al. 2007). The largely reduced expression of IGF-I mRNA in leukocytes and macrophages in the present study together with lowered IGF-I serum levels in our transgenics (Eppler et al. 2007a) may lead to a local IGF-I deficiency which may contribute to explain the reported impaired immune function of GH-transgenic fish. This hypothesis gets support by the lowered IGF-I serum levels and GH receptor mRNA in channel catfish challenged with *Edwardsiella ictaluri* (Peterson et al. 2007) and by down-regulated IGF-I and GH receptor gene expression levels in head kidney of *Enteromyxum leei*-infected gilthead seabream while non-infected individuals showed increased levels (Sitjà-Bobadilla et al. 2008).

Thus, overall GH overexpression does not by nature imply that all organs are positively stimulated. Further studies are needed to evaluate the mechanisms involved in this paradoxical phenomenon. Also the dropped IGF-I serum levels in our GH-transgenic tilapia (Eppler et al. 2007a) somewhat differ from findings in transgenic Coho salmon, where IGF-I serum levels varied between slight enhancement or reduction (Devlin et al. 2000) and elevation in smaller individuals (Raven et al. 2008). Similarly, a positive correlation of body size and plasma IGF-I concentration had been postulated in wild type Coho salmon and tilapia (for references see: Eppler et al. 2007a), but our findings in GH-transgenic tilapia (Eppler et al. 2007a) more support the idea that growth may be less due to endocrine mechanisms but to local IGF-I production as has been demonstrated in transgenic mice where the IGF-I gene was exclusively deleted in liver (Sjögren et al. 1999; Yakar et al. 1999).

In almost all organs investigated, not only IGF-I but also IGF-II mRNA was elevated in the GH-overexpressing tilapia. So far, the role of growth promotion by IGF-II has been mainly attributed to the embryonic or developmental phase (Ayson et al. 2002; Radaelli et al. 2008). Thus, our present results on IGF-II support the hypothesis that life-long exposure to ubiquitous exogenous GH overexpression somehow mimicks the embryonic phase (Caelers et al. 2005). However, an important role of IGF-II in fish life-long growth is suggested (Reinecke and Collet 1998) which is especially important for fish brain with its life-long growth and regeneration capacity. This hypothesis receives support from the numerous IGF-II mRNA-expressing neurons in adult tilapia (Caelers et al. 2003). In GH-overexpressing tilapia, IGF-II mRNA levels gave a higher response than IGF-I mRNA which is similar to findings in GH-injected carp in one study (Tse et al. 2002), while in another IGF-I mRNA was increased but IGF-II mRNA remained unaffected (Vong et al. 2003).

Among all organs investigated, transgenic pituitary was the only one to show no change in IGF-I and IGF-II mRNA. Along with our previous finding that no exogenous salmon but only endogenous tilapia GH mRNA was detectable, this underlines the proposed importance of the local stimulus by the exogenous transgene (Hernández et al. 1997; Caelers et al. 2005). Consistent with findings in salmon (Mori and Devlin 1999), endogenous GH expression was lowered in the pituitary of our GH-transgenic tilapia (Caelers et al. 2005). Along with the dropped IGF-I serum levels (Eppler et al. 2007a) this might indicate that the endocrine IGF-I feedback mechanism on pituitary GH regulation, as is well established throughout evolution (Moriyama et al. 2000; Reinecke et al. 2005) is of minor importance at least in the state of GH redundancy (Eppler et al. 2007a).

To summarize our findings on GH-overexpressing transgenic tilapia, the local production of IGF-I and IGF-II in various organs suggests paracrine and autocrine roles of both IGFs in organ-specific functions in fish. We assume that exposure to extrapituitary GH expression as observed in transgenic fish (Caelers et al. 2005; Raven et al. 2008) not only increases local IGF-I expression in liver and skeletal muscle of GH transgenic tilapia (Eppler et al. 2007a) and coho salmon (Raven et al. 2008) but in numerous tissues promoting growth enhancement of the transgenics. Thus, increased

synthesis of IGF-I which occurred in almost all organs investigated in the present study supports the assumption that paracrine and/or autocrine effects are more relevant for growth than endocrine. Furthermore, we show that permanent high expression of GH in the transgenics not only increases IGF-I but also IGF-II mRNA in liver and most extrahepatic sites indicating that IGF-II is also involved in growth regulation. It is also reasonable that the markedly enhanced expression of liver IGF-II mRNA, that may in particular mimick an early developmental stage, is a further reason for increased growth.

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