EPSA1 and VPF genes expression during embryonic and larval development period of Beluga, *Huso huso*

Taheri Mirghaed, A.*

Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Key words:
Gene expression, larval development, sturgeon

Correspondence
Taheri Mirghaed, A.
Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117092
Fax: +98(21) 66933222
Email: mirghaed@ut.ac.ir

Received: 6 January 2016
Accepted: 15 March 2016

Abstract:

BACKGROUND: The Endothelial PAS domain-containing protein 1 (EPSA1) is the key transcriptional regulator of hypoxic response and Vascular Permeability Factor (VPF) is an important growth factor for vascular development and angiogenesis. **OBJECTIVES:** In the present study, the levels of the EPSA1 coding gene and VPF transcripts were evaluated during Larval development of Beluga, *Huso huso*. **METHODS:** Samples at 12 developmental time-points including 1, 2, 4 days before hatch (eyed eggs), fresh hatched larvae (0), and larvae 1, 3, 6, 10, 15, 20, 25 and 50 days post-hatching were collected and stored in a −80 °C freezer until RNA extraction. Changes in EPSA1 and VPF mRNA expression were studied and differences in normalized mRNA expression levels among the different developmental stages of *H. huso* were analyzed by one-way analysis of variance (ANOVA). **RESULTS:** The transcripts of EPSA1 and VPF were detected in all developmental time-points of *H. huso* from embryos to fingerling fish. Our results revealed that the mRNA expression of EPSA1 and VPF was low during embryonic development and then upregulated significantly at the time of hatch and early larval time-points, whereas in the late larval development stages they started to decline. **CONCLUSIONS:** This study showed that there is an association between the EPSA1 and VPF mRNA expression during larval development of *H. huso*. The up regulation of EPSA1 and VPF transcripts at the time of hatch and during yolk sac fry development of *H. huso* is likely tied to the role of them in vasculogenesis and angiogenesis.

Introduction

The Endothelial PAS domain-containing protein 1 is encoded by the EPAS1 gene. This gene encodes a half of transcription factor involved in the induction of genes regulated by oxygen, which is induced as oxygen levels fall. Endothelial PAS domain-containing protein 1 is the key transcriptional regulator of hypoxic response in both adult and embryonic organisms (Bracken et al., 2003). The transcription factors hypoxia-inducible factor-1 (HIF-1 and EPSA1) is the key regulator responsible for the induction of genes that facilitate adaptation and survival of cells and the whole organism from normoxia to hypoxia (Ke and Costa, 2006). In mammals, expression of at least one hundred genes has been reported to be under the control of HIF-1a and EPSA1 (Rytkonen et al., 2007). A close related protein, EPSA1,

shares 48% amino acid sequence identity with HIF-1. RNA expression patterns have indicated that both HIF-1 and EPSA1 are largely ubiquitously expressed in human and mouse tissues in an oxygen-independent manner (Ke and Costa, 2006). In normoxia, HIF and EPSA protein is transcriptionally inactive and rapidly degraded by the ubiquitin/proteasome pathway. Under hypoxia, however, HIF and EPSA becomes stabilized, translocates into the nucleus and heterodimerises with Aryl Hydrocarbon Nuclear Translocator (ARNT). This transcriptionally active complex then associates with hypoxia response elements (HREs) in the regulatory regions of target genes, binds transcriptional co-activators and induces target gene expression (Bracken et al., 2003).

Vascular Permeability Factor (VPF), a selective mitogen for endothelial cells, is an important growth factor for vascular development and angiogenesis (Liang et al., 2001). It is known that VPF is synthesized by different cell types, including aortic vascular smooth muscle cells, keratinocytes, macrophages and many tumor cells (Dvorak, 1995). VPFs have been found in all vertebrate species that have been examined so far and it has been stated that the sequence and genomic organization of the vertebrate VPF genes is highly conserved between teleost fish and mammals. VPF has been observed in teleost fish (zebrafish, Danio rerio and pufferfish, Fugu rubripes), frogs (Xenopuslaevis), birds (Gallus gallus), and mammals (Holmes and Zachary, 2005). It has also been noted that two isoforms of VPF that differ in the presence of exons 6 and 7, VPF165 and VPF121, are the dominant forms expressed in zebrafish embryo (Liang et al., 2001). In zebrafish, it has been suggested that Vascular Endothelial Growth Factor (VEGF) plays an important role in the vascular development and endoderm morphogenesis (Ober et al., 2004). Moreover, VPF is expressed throughout the zebrafish embryonic development (Liang et al., 1998) and it has been suggested that VPF can not only stimulate endothelial cell differentiation, but also, hematopoiesis in in zebrafish embryo (Liang et al., 2001).

Oxygen tension is a key physiological regulator of VPF-A gene expression. Transcriptional regulation of the VPF gene by hypoxia is mediated by the binding of the transcription factor HIF-1 to the hypoxia responsive enhancer elements (HREs) in its 5′ and 3′ UTRs (Holmes and Zachary, 2005). In mammals, HIF and EPSA signal an increase in VPF, which in turn stimulates the growth of blood vessels (Nikinmaa and Rees, 2005). According to a study on developmental defects in Baltic salmon (*Salmo salar*), it has been suggested that HIF regulates vascular development during normal development, presumably by modulating VPF levels, as in mammals (Vuori et al., 2004; Nikinmaa and Rees, 2005). In lake trout *Salvelinus namaycush*, the yolk sac fry has been shown to have an increasing expression pattern of HIF-1a protein and HIF-1 DNA binding after hatch, however, VPF protein expression was variable between different biological variations (Vouri et al., 2009).

Although the role of VPF in blood vessel formation is well known, it is presently not known how the VPF gene is transcribed during early development of any fish species, especially after hatching. It is also not known whether the transcription of HIFs is associated with VPF transcript during normal development of fish. We addressed these questions in early development of Beluga sturgeon, *Huso huso*, using quantitative real-time PCR (qPCR).

Materials and Methods

Animals and sampling protocol: All fertilized eggs were obtained from an artificially spawned sturgeon broodstock from Shahid Marjani Artificial Sturgeon Propagation and Rearing Center located in Aghalla, Iran. The Beluga Sturgeon larvae hatched seven days after fertilization (Water temperature 18°C),

were reared in ferroconcrete and fiberglass tanks and fed with newly hatched Artemia. The Beluga Larvae started exogenous feeding at day 20. Samples were collected at 12 developmental time-points including 4 days before hatch, 2 days before hatch (eyed eggs), 1 day before hatch, newly hatched larvae (0), and larvae 1, 3, 6, 10, 15, 20, 25 and 50 days post-hatching. All individuals were killed by an overdose of tricaine methanesulfonate (MS-222) and deep-frozen in liquid nitrogen as soon as they were collected and stored in a −80°C freezer until RNA extraction.

Total RNA extraction and cDNA synthesis: The procedure of RNA extraction, control of RNA quality, measuring of RNA concentration and cDNA synthesis have been described by Akbarzadeh et al. (2011). Briefly, whole larvae or eggs were placed in the recommended proportions of Tri Reagent and homogenized using a Qiagen Tissue Lyser. Total RNA was isolated from six individuals at each stage described above (n = 6) using a Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA), treated with Invitrogen DNase I (Invitrogen, CA, USA) and cleaned up using the Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany) in accordance with the manufacturer's instructions. The quality of RNA samples was evaluated by electrophoresis on a 1.5 % agarose gel and their concentration was determined by a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) reading at 260/280 nm. Every sample was measured in duplicate and a mean value was used. 1 µg of total RNA was used to synthesize First-strand cDNAs using a DyNAmoTM cDNA Synthesis Kit (Finnzyme, Espoo, Finland) for RT-PCR, following the manufacturer's instructions and a mixture of oligo-dT as primer.

Primer design: The qPCR primers for ESPA1 and VPF (Kolangi et al., 2013) were designed based on the conserved regions of the sequences in GenBank. Multiple qPCR primer combinations were designed for each gene using Primer3 and tested (Table 1). The specificity and size of the amplicons obtained with primer pairs were checked on a 1.5 % agarose gel. For each gene, qPCR efficiency was also taken into account for choosing the best qPCR primer pair with specific and correct size. Primers for ribosomal protein L6 (RPL6) (used as reference gene for standardization of expression levels) were referenced from Akbarzadeh et al. (2011). The method of PCR efficiency determination has been described in section 2.4.

Quantitative real-time PCR (qRT-PCR): Quantitative PCRs were run on a 7900 HT Fast Real-Time PCR System (Applied Biosystems) with Fermentas Maxima SYBR Green qPCR Master Mix (2×) (Fermentas) and all primers at [100 nM], using standard protocol [initial denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60 °C for 1 min]. All reactions were run in triplicate. The mRNA expression levels of genes were recorded as Ct values that corresponded to the number of cycles at which the fluorescence signal can be detected above a threshold value. Baseline and threshold for Ct calculation were set manually using the SDS RQ manager v 1.2 (Applied Biosystems). Standard curves were constructed from dilution series of pooled cDNA (including seven dilutions from 1/10 to 1/2000), and the PCR efficiency was calculated using the equation $E\% = (10^{1/slope} - 1) \times 100$ (Radonic et al. 2004).

The fold change in relative mRNA expression of EPSA1 and VPF was calculated by the $2^{-\Delta\Delta Ct}$ method of Livak and Schmittgen (2001). The difference between Ct values of the reference genes and the target genes was calculated for each mRNA by taking the mean Ct of triplicate reactions and subtracting the mean Ct of triplicate reactions for the reference RNA measured on an aliquot from the same RT reaction ($\Delta Ct = Ct_{target\ gene} - Ct_{reference\ gene}$). All samples were then normalized to the ΔCt value of a calibrator

sample to obtain a ΔΔCt value ($\Delta Ct_{target} - \Delta Ct_{calibrator}$). Among the developmental time-points, the sample with the lowest Ct value was chosen as the calibrator sample in order to evaluate the putative differential mRNA expression of target gene.

Differences in normalized mRNA expression levels of EPSA1 and VPF among the different developmental stages of *H. huso* were analyzed by one-way analysis of variance (ANOVA), followed by a Tukey's HSD post hoc analysis for multiple comparisons. Differences were considered statistically significant at $p<0.05$. SPSS (version 18.0) software was used for statistical analysis.

Results

Relative transcription pattern of EPSA1: The transcript of EPSA1 was detected in all developmental time-points of *H. huso* from embryos to fingerling fish (Fig. 1 A&B). Changes in normalized EPSA1 transcription by using the RPL6 over development were statistically significant over time ($p<0.05$). Both EPSA1 (Fig. 1) relative mRNA levels were significantly upregulated from hatching time to late larval stages ($p<0.05$). The transcript levels of EPSA1 displayed a significant decrease from feeding time (20dph) to fingerling fish.

Relative transcription pattern of VPF: Figure 2 shows the result for the quantification of the developmental expression of VPF in *H. huso*. Similar to EPSA1, the mRNA of VPF could be detected as early as embryonic time (d-4). Changes in normalized VPF expression over embryonic and larval development of Beluga were statistically significant over time ($p<0.05$). After low expression during the embryonic stage (4, 2 and 1 days before hatch) the transcript levels of VPF displayed an increasing trend from hatching time (Fig. 2).

The relationship between the transcription patterns of EPSA1 and VPF: To show the relationship between the transcription of EPSA1 and VEGF, maximal mRNA values were adjusted to 1, and other values were fractioned to indicate the proportion of the transcription levels compared to maximum. The results show a similar pattern was observed for EPSA1 and VPF transcripts in embryonic and larval development. The mRNA expression of both genes was low during embryonic development and then upregulated during hatching and early larval stages, whereas in the late larval and juvenile stages it remained fairly constant.

Discussion

In this study, we have found a direct relationship between the EPSA1 and VPF mRNA expression during normal development of *H. huso*. This is the first report of the association of HIFs and VPF mRNA expression during larval development of fish in a normal oxygen environment. The levels of EPSA1 and VPF transcripts were elevated significantly during and after hatching time and remained fairly constant in the late larval and juvenile stages.

The upregulation of EPSA 1 and VPF transcripts at the time of hatch and during yolk sac fry development of *H. huso* observed in the present study is likely to be tied to the role of EPSA1 and VPF in organogenesis, vasculogenesis and angiogenesis. It is well known that regulation of EPSA1, VPF and VPF receptor in the embryo is crucial to angiogenesis and, therefore, to organogenesis and survival (Bonventre et al., 2011). The role of EPSA on the regulation of the VPF gene and vascular development in hypoxia condition has been well studied (e.g. Levy et al., 1995; Forsythe et al. 1996; Miquerol et al., 2000; Holmes and Zachary, 2005; Kallergi et al., 2009). EPSA is an important regulator of hypoxia responses, including vascularization and erythropoiesis, and is required for normal development, including angiogenetic, hematopoietic, and neural development in mammalian embryos

Table 1. qPCR primer used in this study.

Primer name	Seq	Amplicon size	Accession number/Ref.
ESPA1 For	GAAGGTCCTGCACTGCACT		JQ027715.1
ESPA1 Rev	CTTGGTGCACAAGTTCTGGT		JQ027715.1
RPL6 For	GTGGTCAAACTCCGCAAGA		Akbarzadeh et al. (2011).
RPL6 Rev	GCCAGTAAGGAGGATGAGGA		Akbarzadeh et al. (2011).
VPF For	GCCTTCATGTGTACCACTCATG		Kolangi et al. (2013)
VPF Rev	GGTCTGCATTCACATGTACTGTG		Kolangi et al. (2013)

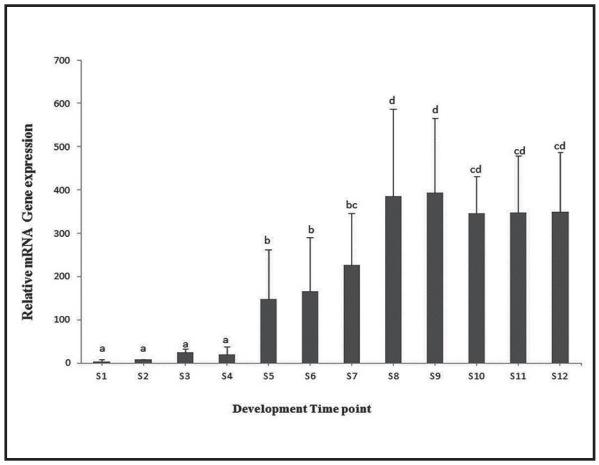

Figure 1. Relative expression levels of EPSA1 mRNA normalized using RPL6 during early development of Beluga. S1: 4 days before hatch; S2: 2 days before hatch; S3: 1 day before hatch; S4: newly hatched (day 0); S5: 1 day post-hatch (1 dph), S6:3 dph; S7: 6 dph; S8: 10 dph; S9:15 dph; S10: 20 dph; S11: 25 dph; S12: 50 dph. The values are represented as mean ± S.D. (n = 6). Statistical significance of differences of the normalized EPSA 1 data between groups was analyzed using one-way ANOVA and Tukey's multiple-comparison test. Bars with different letters are significantly different. $p<0.05$ was taken to show significant differences.

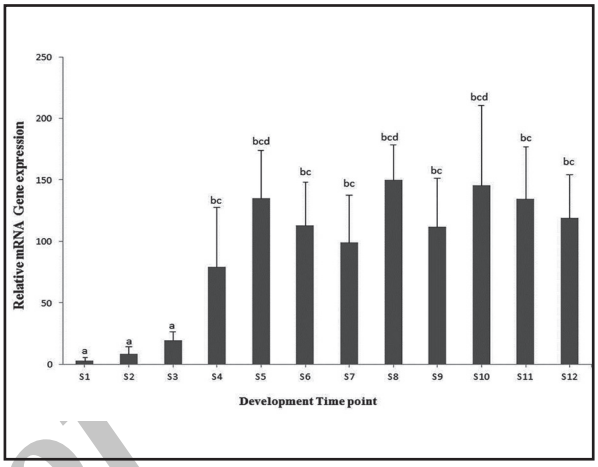

Figure 2. Relative expression levels of VPF mRNA normalized using RPL6 during early development of Beluga. S1: 4 days before hatch; S2: 2 days before hatch; S3: 1 day before hatch; S4: newly hatched (day 0); S5: 1 day post-hatch (1 dph), S6:3 dph; S7: 6 dph; S8: 10 dph; S9:15 dph; S10: 20 dph; S11: 25 dph; S12: 50 dph. The values are represented as mean ± S.D. (n = 6). Statistical significance of differences of the normalized VPF data between groups was analyzed using one-way ANOVA and Tukey's multiple-comparison test. Bars with different letters are significantly different. $p<0.05$ was taken to show significant differences.

(Vuori et al., 2004). In mammals, abnormal blood vessel development and vascularization are observed when embryos lacking a single VPF allele or HIFs are disturbed (Carmeliet et al., 1996; Ryan et al., 1998). In Baltic salmon, Vuori et al. (2004) found that both HIF-1 and VPF down-regulated in Baltic salmon suffer from abnormally high yolk-sac fry mortality (M74-syndrome). They suggested that HIF-1 regulates vascular development during normal development by modulating VPF levels. In lake trout, the expression of HIFs protein and HIF-1 DNA binding showed an increasing pattern after hatch and during yolk sac fry (Vouri et al., 2009).

Interestingly, the upregulation of VPF gene was observed at time of hatch and during yolk sac fry of *H. huso*. VPF has emerged as the single most important regulator of blood vessel formation (Holmes and Zachary, 2005). Various processes of early stage vascular development including vasculogenesis, large vessel formation (e.g. of the dorsal aorta), capillary sprouting, and the remodeling of the yolk sac vasculature are affected by VPF (Breier, 2000). Indeed, the expression of the VPF has been thoroughly studied during embryonic development. However, little is known about the role of VPF on vascular development at the time of hatching and during larval development of fish.

In zebrafish, overexpression of VPF in the embryo indicates a stimulation of both endothelial and hematopoietic lineages. It has also been demonstrated that VPF can stimulate hematopoiesis in zebrafish by promoting the formation of terminally differentiated red blood cells (Liang et al., 2001). Hendon et al. (2008) studied the effects of exposure to pyrene in the early life-stages of ship head minnow, *Cyprinodon variegatus*, and observed that the VPF gene is transcribed under conditions where the external environment is normoxic, and suggested that the oxygen tension in developing tissues is reduced to a level that prevents HIF degradation resulting in VPF transcription. In mouse, VPF was detected from embryonic day 7 in the extra-embryonic and embryonic endoderm, and by day 8 it is present at high levels in the trophoblast surrounding the embryo and in the embryonic myocardium, gut endoderm, embryonic mesenchyme and amniotic ectoderm. VPF expression declines in most tissues in the weeks after birth and is relatively low in most adult organs, except in a few vascular beds, including those of the brain choroid plexus, lung alveoli, kidney glomeruli and heart (Holmes and Zachary, 2005). VPF gene expression is also upregulated by a variety of growth factors and cytokines, including PDGF-BB, TGF-ß, basic fibroblast growth factor (FGF-2), interleukin-1ß and interleukin-6, some of which can act synergistically with hypoxia (Holmes and Zachary, 2005).

Our results indicate that VPF is involved in development of the vascular system of *H. huso* larvae in normoxic condition. It has been suggested that organogenesis is closely linked to vascular development and formation of the vertebrate closed circulatory system which involves both vasculogenesis and angiogenesis (Bonventre et al., 2011). During yolk sac fry development of fish, new capillaries are formed and larval-type red blood cells and hemoglobins are replaced by adult types (Iuchi and Yamamoto 1983; Wells and Pinder 1996). The upregulation of VPF can therefore be considered a major factor by which the formation of blood vessels and cells is regulated.

The results of the present study also showed a marked upregulation of EPSA1 transcript at the time of hatch and larval development of *H. huso*. EPSA1 is a closely related protein complex which is regulated by cellular oxygen concentrations in a similar fashion. These hypoxia inducible factors activate transcription of target genes in response to hypoxia (Blancher et al., 2000; Losso and Bawadi, 2005). It has been demonstrated that EPSA1 is largely ubiquitously expressed in an oxygen-independent manner (Bracken et al., 2003). In normoxia, HIF protein is transcriptionally inactive and rapidly degraded by the ubiquitin/proteasome pathway (Bracken et al., 2003). However, Hypoxic conditions inhibit this degradation, which allows HIFs to accumulate in the cell (Kajimura et al., 2006). Interestingly, however, there are several reports of HIF protein expression at normoxia in which imply more diverse roles for HIF than solely regulating a hypoxic response (Bracken et al., 2003). EPSA1 is required for normal embryogenesis because it is central to oxygen homeostasis. HIF-1 and EPSA1 knockout mice died early or had syndromes of multiple organ pathology that included retinopathy, cardiac hypertrophy, mitochondrial abnormalities, hypoglycemia, altered Krebs cycle, and several biochemical abnormalities (Losso and Bawadi, 2005). The up-regulation of EPSA1 gene, especially at the time of hatch during development of *H. huso* in normoxic condition reveals the possible importance of HIF signaling during this period.

Conclusion: In conclusion, this is the first report of the association of EPSA1 and VPF mRNA expression during larval development of fish in a normal oxygen environment. Our data revealed that the levels of EPSA1 and VPF transcripts were elevated significantly during and after hatching time and remained fairly constant in the late larval and fingerling

stages. The upregulation of EPSA1 and VPF transcripts at the time of hatch and during yolk sac fry development of *H. huso* observed in the present study is most likely tied to the role of EPSA1 and VPF in organogenesis, vasculogenesis and angiogenesis.

Acknowledgments

We would like to thank the staffs of Sturgeon Propagation and Rearing Center of Shahid Marjani (Gorgan, Iran) for their kind help. Special thanks are forwarded to Mr. Pasandi and Mr. Ghamsari for their guides and assistances.

References

1. Akbarzadeh, A., Farahmand, H., Mahjoubi, F., Nematollahi, M.A., Leskinen, P., Rytkonen, K., Nikinmaa, M. (2011) The transcription of L-gulono-gamma-lactone oxidase, a key enzyme for biosynthesis of ascorbic acid, during development of Persian sturgeon *Acipenser persicus*. Comp Biochem Physiol B. 158: 282-288.
2. Blancher, C., Moore, J.W., Talks, K.L., Houlbrook, S., Harris, A.L. (2000) Relationship of Hypoxia-Inducible Factor (HIF)-1a and HIF-2a expression to vascular endothelial growth factor induction and hypoxia survival in human breast cancer cell lines. Cancer Res. 60: 7106-7113
3. Bonventre, J.A., White, L.A., Cooper, K.R. (2011) Methyl tert butyl ether targets developing vasculature in zebrafish (*Danio rerio*) embryos. Aquat Toxicol. 105: 29-40.
4. Bracken, C.P., Whitelaw, M.L., Peet, D.J. (2003) The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. Cell Mol Life Sci. 60: 1376-1393.
5. Breier, G. (2000) Angiogenesis in embryonic development: A Review. Placenta. 14: 11-15.
6. Carmeliet, P., Ferreira, V., Breier, G., Pollefeyt, S., Kieckens, L., Gertsenstein, M., Fahrig, M., Vandenhoeck, A., Harpal, K., Eberhardt, C., Declercq, C., Pawling, J., Moons, L., Collen, D., Risau, W., Nagy, A. (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 380: 435-439.
7. Dvorak, H.F., Brown, L.F., Detmar, M., Dvorak, A.M. (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol. 146: 1029-1039.
8. Forsythe, J.A., Jiang, B.H., Iyer, N.V., Agani, F., Leung, S.W., Koos, R.D., Semenza, G. (1996) Activation of vascular endothelial growth factor gene transcription by Hypoxia-Inducible Factor 1. Mol Cell Biol. 16: 4604-4613.
9. Hendon, L.A., Carlson, E.A., Manning, S., Brouwer, M. (2008) Molecular and developmental effects of exposure to pyrene in the early life-stages of *Cyprinodon variegatus*. Comp Biochem Physiol C. 147: 205-215.
10. Holmes, D.I., Zachary, I. (2005) The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol. 6: 209.
11. Kajimura, S., Aida, K., Duan, C. (2006) Understanding Hypoxia-Induced Gene Expression in Early Development: In Vitro and In Vivo analysis of hypoxia-inducible factor 1-regulated Zebra fish insulin-like growth factor binding protein 1gene expression. Mol Cell Biol. 26: 1142-1155.
12. Kallergi, G., Markomanolaki, H., Giannoukaraki, V., Papadaki, M.A., Strati, A., Lianidou, E.S., Georgoulias, V., Mavroudis, D., Agelaki, S. (2009) Hypoxia-inducible factor-1α and vascular endothelial growth factor expression in circulating tumor cells of breast cancer patients. Breast Cancer Res. 11: R84-12.
13. Ke, Q., Costa, M. (2006) Hypoxia-Inducible Factor-1 (HIF-1). Mol Pharmacol. 70: 1469-1480.
14. Kolangi Miandare, H., Farahmand, H., AKbarzaeh, A., Ramzanpour, S., Kaiya, H., Mi-

yazato, M., Rytkonen, K.T., Nikinmma, M. (2013) Developmental transcription of gene putatively associated with growth in two sturgeon species of different growth rate. Gen Comp Endocr. 182: 41-47.

15. Levy, A.P., Levy, N.S., Wegner, S., Goldberg, M.A. (1995) Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. J Biol Chem. 270: 13333-13340.
16. Liang, L., Xu X., Chin A.J., Balasubramaniyan, N.V., Teo, M.A.L., Lam, T.J., Weinberg, E.S., Ge, R. (1998) Cloning and characterization of vascular endothelial growth factor (VEGF) from zebrafish, *Danio rerio*. Biochim Biophys Acta. 1397: 14-20.
17. Liang, L., Chang, J.R., Chin, A.J., Smith, A., Kelly, C., Weinberg, E.S., Ge, R. (2001) The role of vascular endothelial growth factor (VEGF) in vasculogenesis, angiogenesis, and hematopoiesis in zebrafish development. Mech Dev. 108: 29-43.
18. Livak, K.J., Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25: 402-408.
19. Losso, J.N., Bawadi, H.A. (2005) Hypoxia Inducible factor pathways as targets for functional foods. J Agric Food Chem. 53: 3751-3768.
20. Iuchi, I., Yamamoto, M. (1983) Erythropoiesis in the developing rainbow trout, *Salmo gairdneri* irideus: histochemical and immunochemical detection of erythropoietic organs. J Exp Zool. 226: 409-417.
21. Miquerol, L., Langille, B.L., Nagy, A. (2000) Embryonic development is disrupted by modest increases in vascular endothelial growth factor gene expression. Development. 127: 3941-3946.
22. Nikinmaa, M., Rees, B.B. (2005) Oxygen-dependent gene expression in fishes. Am J Physiol Regul Integr Comp Physiol. 288: 1079-1090.
23. Ober, E.A., Olofsson, B., Makinen, T., Jin, S.W., Shoji, W., Koh, G.Y., Alitalo, K., Stainier, D.Y.R. (2004) VEGFc is required for vascular development and endoderm morphogenesis in zebrafish. EMBO Rep. 5: 78-84.
24 Radonic, A., Thulke, S., Mackay, I.M., Landt, O., Siegert, W., Nitsche, A. (2004) Guideline to reference gene selection for quantitative real-time PCR. Biochem Biophys Res Commun. 313: 856-862.
25. Ryan, H.E., Lo, J., Johnson, R.S. (1998) HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J. 17: 3005-3015.
26. Rytkonen, K.T., Vuori, K.A.M., Primmer, C.R., Nikinmaa, M. (2007) Comparison of hypoxia-inducible factor-1 alpha in hypoxia-sensitive and hypoxia-tolerant fish species. Comp Biochem Physiol D. 2: 177-186.
27. Vuori, K.A., Soitamo, A., Vuorinen, P.J., Nikinmaa, M. (2004) Baltic salmon (*Salmo salar*) yolk sac fry mortality is associated with disturbances in the function of hypoxia-inducible transcription factor (HIF-1 alpha) and consecutive gene expression. Aquat Toxicol. 68: 301-313.
28. Vuori, K.A., Paavilainen, T., Nikinmaa, M. (2009) Molecular markers of yolk sac fry development in nine families of lake trout. J Aquat Anim Health. 21: 279-289.
29. Wells, P., Pinder, A. (1996) The respiratory development of Atlantic salmon, I. Morphometry of gills, yolk sac, and body surface. J Exp Biol. 199: 2725-2736.

مجله طب دامی ایران، ۱۳۹۵، دوره ۱۰، شماره۲، ۱۵۰-۱۴۳

بیان ژن‌های EPSA۱ و VPF در طی دوره تکامل جنینی و لاروی فیل ماهی (*Huso huso*)

علی طاهری میرقائد*

گروه بهداشت و بیماریهای آبزیان، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(دریافت مقاله: ۱۶ دی ماه ۱۳۹۴، پذیرش نهایی: ۲۵ اسفند ماه ۱۳۹۴)

چکیده

زمینه مطالعه: ژن EPSA۱ (Endothelial PAS domain-containing protein ۱) یک تنظیم کننده رونویسی تحت تأثیر شـرایط هایپوکسـی بوده و ژن VPF (Vascular Permeability Factor) بعنوان یک فاکتور مهم برای رشـد و تکامل عروق خونی می‌باشد. **هدف:** در این مطالعه سطوح بیان ژن‌های EPSA۱ و VPF در دوره تکامل لاروی فیل ماهی مورد بررسی قرار گرفت. **روش کار:** نمونه برداری از ۱۲ مرحله تکاملی شامل ۱، ۲ و ۴ روز قبل از تفریخ تخم، لارو های تازه تفریخ شده و لاروهای ۱، ۳، ۶، ۱۰، ۱۵، ۲۰، ۲۵ و ۵۰ روز پـس از تفریـخ صورت پذیرفت. نمونه‌ها تا زمان انجام مراحل کار و اسـتخراج RNA در فریزر ۸۰°C- نگهداری شدند. سپس تغییرات میزان بیان ژن‌های EPSA و VPF مورد مطالعه قرار گرفت و تفاوت بین بیان ژن‌های مذکور در مراحل مختلف تکاملی توسـط آنالیز واریانس یک طرفه (one-way ANOVA) مورد بررسی قرار گرفت. **نتایج:** بیان ژن‌های ژن‌های EPSA و VPF در تمامی مراحل تکاملی جنینی و مراحل ابتدایی لاروی صورت پذیرفت. نتایج نشـان داد که بیان ژن‌های ژن‌های EPSA و VPF در دوره جنینی پایین بوده و سپس در زمان تفریخ و مراحل ابتدایی تکامل لاروی افزایش در بیان مشاهده شد درحالیکه با افزایش سن لارو بیان ژن‌های مذکور روند رو به کاهش داشت. **نتیجه گیری نهایی:** این مطالعه نشان می‌دهد که ارتباط تنگاتنگی بین ژن‌های ژن‌های EPSA و VPF در مراحل تکامل لاروی فیل ماهی وجود دارد و افزایش بیان ژن‌ها مذکور در مراحل ابتدای تفریخ و مراحل تکاملی لاروی جذب زرده فیل ماهی، نشان دهنده اهمیت آن در تکامل رگزایی می‌باشد.

واژه‌های کلیدی: بیان ژن، تکامل لاروی، ماهی خاویاری

*) نویسنده مسؤول: تلفن: ۶۱۱۱۷۰۹۲ (۲۱)۹۸+ نمابر: ۶۶۹۳۳۲۲۲ (۲۱)۹۸+ Email: mirghaed@ut.ac.ir