

**EXPRESSION STUDIES OF THE *VEGF-A* AND *FLK-1* (VEGF
RECEPTOR) GENES IN GRASS CARP**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Eutrophication is a worldwide problem and causes massive fish kills due to oxygen depletion (aquatic hypoxia). Grass carp (*Ctenopharyngodon idellus*) is a hypoxia-tolerant fish and can withstand severe hypoxia (0.5 mg/L DO) for prolonged periods. In mammals, vascular endothelial growth factor (*vegf-A*) and VEGF-receptor 2 (*flk-1*) genes play important roles in angiogenesis and are upregulated to enhance O₂ delivery under low oxygen conditions (Veikkola, 2000; Ferrara, 2001). It has been demonstrated that *vegf-A* expression is controlled by the HIF-1 (Hypoxia-inducible factor 1) transcription factor under hypoxia (Levy et al., 1995) but the regulatory effect of HIF-1 on *flk-1* expression is unconfirmed. Moreover, VEGF (Wilting et al., 1996) and hypoxia (Waltenberger et al., 1996) are known to potentiate the expression of *flk-1*. In lower vertebrates, the relationship between *vegf-A* and *flk-1* expression and the effect of hypoxia on these genes have not been studied.

In an attempt to investigate the *in vivo* responses of the *vegf-A* and *flk-1* genes to hypoxic stress in the grass carp, and their physiological roles in hypoxia tolerance, we have isolated a 355-bp *vegf-A* and 763-bp *flk-1* cDNA fragment from grass carp kidney total RNA by degenerate RT-PCR. The RT-PCR products were cloned into plasmid vectors and were confirmed by DNA sequencing to represent the grass carp *vegf-A* and *flk-1* cDNAs. These cDNA fragments were radiolabelled with ³²P-dCTP and were used as probes to examine the *in vivo* tissue distribution and expression patterns of the genes under normoxic and hypoxic conditions. Total RNA was extracted from 5 different tissues of grass carps that were maintained under normoxic (7.0 mg/L DO) or hypoxic (0.5 mg/L DO) conditions for 4 and 96 hours and blotted onto Nylon membranes for Northern hybridization.

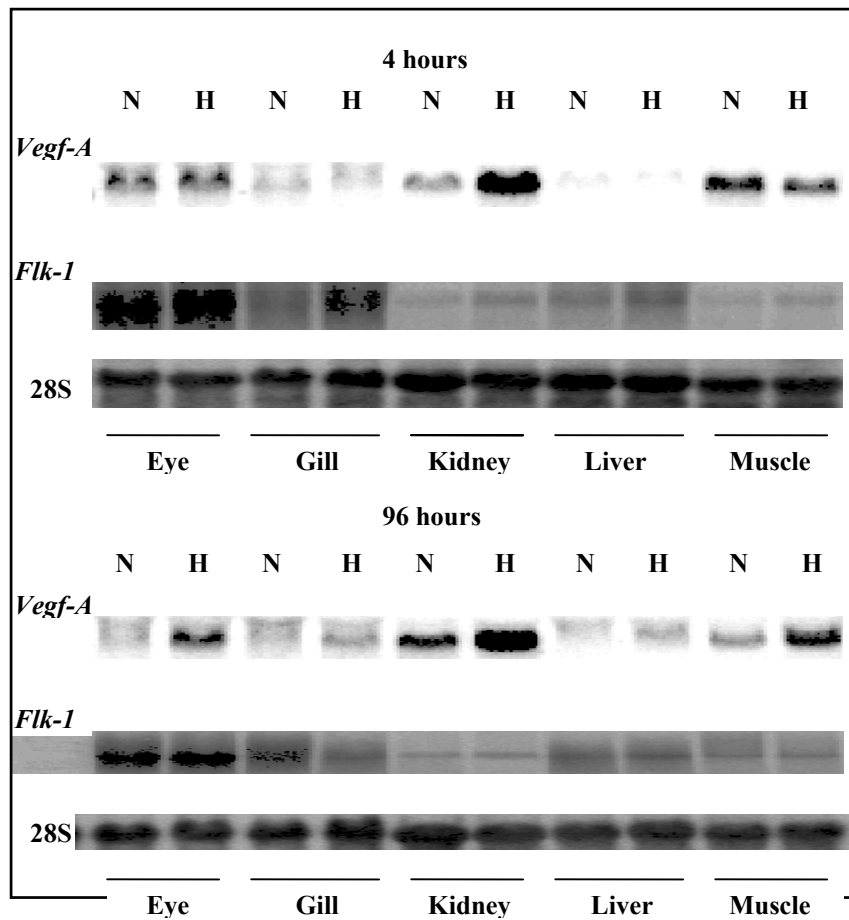


Figure 1. Northern blot analysis of *Vegf-A* and *Flk-1*.

N: Tissues from grass carp under normoxia (7.0 mg/L DO);
H: Tissues from grass carp under hypoxia (0.5 mg/L DO)
Expression was normalized against the 28S ribosomal RNA.

The *vegf-A* and *flk-1* transcripts are approximately 3.0 and 7.0 kb in size, respectively, and are in agreement with those reported in other vertebrate species. Amongst the 5 different tissues examined by northern hybridization, muscle (4 hrs) and kidney (96 hrs) showed the highest *vegf-A* expression under normoxic conditions; with the lowest expression observed in liver and

gill (Figure 1). In contrast, *flk-1* is highly expressed in eye, and only low mRNA levels were detected in gill, kidney, liver and muscle.

Expression of *vegf-A* was markedly upregulated in the fish kidney during short term (4 hrs) and prolonged (96 hrs) hypoxia, while upregulated expression in eye and muscle was observed only upon prolonged hypoxia (96 hrs). Expression of *vegf-A* was seemingly unaffected by hypoxia in gill and liver. Although *flk-1* is most highly expressed in eye, its expression is not induced in this tissue by short term and prolonged hypoxia. Because *flk-1* is expressed at very low levels in other fish organs, gene activation in response to hypoxia was observed in the gill and kidney only when the autoradiogram was exposed for an additional 24 hrs (data not shown).

In conclusion, *vegf-A* and *flk-1* are differentially regulated by hypoxia in different fish organs. Only in the fish kidney are the *vegf-A* and *flk-1* genes co-induced by hypoxia, albeit at different levels.

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