

## IN SITU DEMONSTRATION OF TYPE I-III INTERMEDIATE FILAMENT EXPRESSION IN THE COMMON CARP

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### Introduction

The mammalian intermediate filaments (IF) are a multigenic family of 10 nm cytoskeletal polypeptides that are generally classified into five major types according to their amino acid-sequence homologies, isoelectric points and tissue expression patterns (Franke et al., 1982; Lazarides, 1982; Franke, 1987; Steinert and Roop, 1988). Among these IF types, the cytokeratins are a heterogeneous group encoded by a set of closely related genes and classified according to their biochemical similarities (Moll et al., 1982). According to this classification scheme, the type I and type II IF are composed of the acidic, low molecular weight (CK 9-19) and the basic, high molecular weight (CK 1-8) cytokeratins, respectively (Moll et al., 1982; Quinlan et al., 1985). The type III IF include vimentin, desmin, glial fibrillary acidic protein and peripherin that are expressed in mesenchymal, muscle, astroglial and neuronal cells, respectively (Traub, 1985). Vimentin and desmin are structurally similar yet immunologically distinct IF that are each encoded by a single gene (Quax et al., 1984) although mRNA variants with differential tissue expression patterns have previously been demonstrated (Dodemont et al., 1982; Zehner and Paterson, 1983).

At the cellular level of organization, the mammalian IF are differentially expressed in various combinations dependent on the origin of the cell and the state of cellular differentiation (Franke et al., 1982). Accordingly, the cytokeratins are generally expressed in epithelial cell types (Moll et al., 1982; Fuchs et al., 1987) whereas vimentin and desmin are expressed in mesenchymal and muscle cells, respectively (Traub, 1985). Furthermore, the cytokeratins can be subclassified according to their expression in simple versus complex epithelium (Sun et al., 1984; O'Guin et al., 1987). Recognition of these differential expression patterns has been exploited as an adjunct diagnostic tool in the differentiation of epithelial and non-epithelial cell types in developmental and pathological processes (Osborn and Weber, 1983; Moll, 1989).

Previous nucleic acid sequencing and hybridization studies have shown that the IF have been highly conserved during vertebrate evolution (Fuchs et al., 1981; Fuchs and Marchuk, 1983; Quax et al., 1984). Presumably, this phylogenetic persistence would infer the expression of shared structural similarities among the vertebrate IF recognizable by heterologous antibodies. In this context, the common carp (*Cyprinus carpio*) was selected as a teleost model in the present study to evaluate the immunological cross-reactivity and tissue distribution patterns of vimentin, desmin and the cytokeratins using heterologous antibodies as detection reagents.

### Materials and Methods

Tissues from sexually mature, ornamental (koi) common carp with a length of 25-38 cm and a weight of 1-2 kg were fixed in 10% neutral-buffered formalin, 100% ethanol and methacarn. Histologic sections of all tissues were cut to 4  $\mu$ m prior to staining. Six heterologous antibodies were evaluated using a streptavidin-biotin-peroxidase complex (ABC) detection system. Anti-cytokeratin antibodies included the murine monoclonal antibodies AE1 and AE3 (BioGenex Laboratories, San

Ramon, CA) specific for human type I (CK 10, 14-16 and 19) and type II (CK 1-8) cytokeratins. Anti-mammalian vimentin antibodies included the rabbit polyclonal antibody 68-121 (ICN Biomedicals, Inc., Costa Mesa, CA) and the murine monoclonal antibodies Vim 3B4 (Dako Laboratories, Carpinteria, CA) and V9 (BioGenex Laboratories). Desmin cross-reactivity was evaluated using the mammalian anti-desmin murine monoclonal antibody 33 (BioGenex Laboratories). The dilutions used were as follows: 68-121 (1:400), Vim 3B4 (1:200), V9 (1:50) and 33 (1:100, 1:200). The antibodies AE1 and AE3 were used undiluted as provided by the manufacturer.

## Results

Results for the common carp tissues fixed in 100% ethanol and methacarn were similar. Specifically, the AE3 antibody generally provided a discrete and intense signal against a variety of epithelial tissues including the epithelium of the integument, gill, oropharynx, esophagus, intestine, bile and pancreatic ducts, bile canaliculi, swim bladder and renal tubules. The AE3 antibody also stained the arterial and venous endothelium, ependymal cells including the choroid plexus epithelium and the specialized lens-fiber epithelium. Cytokeratin detection using AE3 was not restricted to epithelial tissue but also included a variety of non-epithelial tissue including the fibrous connective tissue, chondrocytes, testicular interstitial myoid cells, renal interstitial stromal cells, mesangial cells, meninx, glial cells of the optic nerve, chromatophores, cuboidal spermatogonia lining the seminiferous tubules and the the oogonia.

The AE1 antibody resulted in a staining pattern similar to that achieved with AE3 with a few notable exceptions. There was a weak and variable staining of the vascular endothelium, the bile canaliculi and the glomerular epithelial and mesangial cells and a generalized less intense staining of all tissues relative to AE3. However, the skeletal muscle and myocardium stained with the AE1 antibody.

Vimentin localization using the 68-121 polyclonal antibody generally resulted in a discrete staining of a variety of epithelial and non-epithelial tissues. The former included the epithelium of the integument, gills, oropharynx, esophagus, intestine, swim bladder, biliary and pancreatic ducts, exocrine pancreas, renal tubules, ependyma, choroid plexus, lens fibers and the retinal pigment layer. As expected, the 68-121 antibody stained a variety of non-epithelial tissues including fibrous connective tissue, various muscle fiber types, chondrocytes, lymphohematopoietic cells, mesangial cells, chromatophores, pillar cells and the meninx. Staining of the glial cells of the optic nerve and neurons of the brain was weak or absent. There was also a diffuse, weak staining of the various retinal layers associated with a diffuse, intense staining of the cells and fibers of the retinal ganglion layer.

Staining with the V9 monoclonal antibody was restricted in comparison to the 68-121 antibody. This restricted staining included an intense staining of the cells and fibers of the retinal ganglion layer and a diffuse, weak to moderate staining of the lens epithelium. There was also an intense staining of the choroid plexus epithelium and a variable weak staining of the meninx. The basal lamina of the integument also displayed a fine, discrete, moderate to intense staining. In contrast, staining with the Vim 3B4 monoclonal antibody was uniformly negative. Desmin cross-reactivity using the 33 monoclonal antibody was negative except for the restricted, diffuse, intense staining of the lens epithelium.

Formalin fixation resulted in a less intense staining or absence of staining that was often associated with considerable background staining. Therefore, formalin was considered the least desirable fixative.

## Discussion

Results of the present study demonstrate that heterologous antibodies to mammalian type I, II and III IF recognize homologous proteins in the common carp. This immunological cross-reactivity of the IF between taxonomically distant species is consistent with previous studies that have demonstrated the conservation of IF genes during vertebrate evolution (Fuchs et al., 1981; Fuchs and Marchuk, 1983; Quax et al., 1984). The phylogenetic persistence of genetic and structural homologies among the vertebrate IF in association with the demonstrated immunological cross-reactivity using heterologous antibodies (Franke et al., 1979; Nelson and Traub, 1982) suggests that the IF genes have been derived from a common ancestral gene (Fuchs and Marchuk, 1983; Quax et al., 1983;

Marchuk et al., 1984) that may have coincided with the origination of the chordates or deuterostomes (Fuchs and Marchuk, 1983; Weber et al., 1988, 1989; Riemer et al., 1992). Despite the phylogenetic persistence of IF during vertebrate evolution, previous investigations of teleost IF have indicated that the biochemical properties and tissue expression patterns in these species may be fundamentally different than mammals.

The present findings were consistent with the cytokeratin expression pattern demonstrated in the rainbow trout (Markl and Franke, 1988; Markl et al., 1989) and goldfish (Giordano et al., 1990). In contrast, studies in the northern pike using heterologous anti-cytokeratin antibodies described a restricted epithelial staining pattern (Thompson et al., 1987) similar to the results reported for striped bass and medaka tissues (Bunton, 1993).

An immunohistochemical staining pattern using heterologous anti-vimentin antibodies has been reported for various mesenchymal tissues in teleosts including fibrous connective tissue and muscle in the northern pike (Thompson et al., 1987) and medaka (Bunton 1995) similar to the pattern in mammals. However, vimentin cross-reactivity using the 68-121 antibody in the present study was not limited to mesenchymal tissues but also included epithelial tissues. The restricted expression of a vimentin homolog in the integument, lens and whole blood of the rainbow trout suggested that vimentin may be a minor and highly restricted cytoskeletal component in teleosts with further speculation that a cytokeratin/vimentin shift may have occurred during vertebrate evolution (Markl et al., 1989; Markl, 1991). Findings of vimentin variants with a differential tissue expression pattern in the goldfish further indicate that vimentin may be a minor and highly restricted cytoskeletal component in teleosts (Glasgow et al. 1994).

This variation in results among teleost species may be attributed to distinct species characteristics or to differences in the specificity of the primary antibody. In this context, the relatively diverse tissue distribution pattern observed using the 68-121 polyclonal antibody versus the V9 monoclonal antibody would be expected due to the inherent polyspecificity of polyclonal antibodies versus the characteristic monospecificity of monoclonal antibodies. This explanation is also consistent with findings in the rainbow trout that several complex epithelial cytokeratins express both common and unique epitopes (Markl et al., 1989). Regardless, this phenomenon is most likely a manifestation of the IF subunit-peptide structure that is comprised of central domains with similar amino-acid homologies and non-homologous head and tail domains that flank the central domains and confer the antigenic and functional specificities among the individual IF proteins (Parry, 1990; Steven, 1990). Therefore, the detection of IF in teleosts using heterologous antibodies only demonstrates the conservation of common epitopes during vertebrate evolution but does not permit the inference that the recognized polypeptides are equivalent or similar in such taxonomically distant species. However, differences in technique do not adequately explain the variability among the teleosts using common antibodies that may ultimately be due to species differences. Further research is therefore necessary to determine the extent of differences in IF expression among teleost species within and between taxa and to determine the phylogenetic branch points for these differences.

The results of the present study indicate that the cytokeratin and vimentin IF in the common carp exhibit a diverse epithelial and non-epithelial tissue distribution pattern. These findings are consistent with those reported in other teleosts but fundamentally conflict with the IF expression patterns in mammals. These results suggest caution in the indiscriminate use of heterologous antibodies as a method for the determination of cell histogenesis in teleosts. Interpretation of results using particular heterologous antibodies in teleosts should be based on prior normal tissue distribution studies in these species and not on the patterns known to occur in mammals.

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