Molecular markers of cancer in cartilaginous fish: immunocytochemical study of PCNA, *p-53*, *myc* and *ras* expression in neoplastic and hyperplastic tissues from free ranging blue sharks, *Prionace glauca* (L.)

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Abstract

Archival formalin-fixed tissues from wild-caught adult blue sharks, *Prionace glauca* (L.), were used for immunocytochemical detection of proliferating cell nuclear antigen (PCNA), two oncoproteins from the oncogenes *c-myc* and *pan-ras*, and a protein product from the tumour suppressor gene *p-53*. All sharks were caught during summer months between 2000 and 2006 by recreational fishermen off the USA coast in the northwestern Atlantic. The sharks were necropsied on landing and selected organ samples were collected into elasmobranch formalin and processed for paraffin embedding and light microscopy. Paraffin-embedded sections from collected tissue were both stained with haematoxylin and eosin and processed by immunocytochemical techniques using antibodies raised against the PCNA, p-ras, c-myc and p-53 proteins. The lesions examined in this study included two well differentiated adenomatous gastric polyps, a testicular capsular mesothelioma, a gingival fibropapilloma with elements of ameloblastoma, three liver tumours, two pericardial fibropapillomas and six cases of proliferative serositis (pericarditis and peritonitis). Normal and hyperplastic tissues from blue sharks, and human neoplastic tissues served as negative and positive controls, respectively. We detected upregulation of PCNA in many neoplastic, one dysplastic and in some hyperplastic lesions, and positive p-ras and c-myc signals in some of the neoplastic lesions. None of the examined tissues showed positive p-53 signalling. This is the first literature report on immunocytochemical detection of molecular markers of cancer in sharks and in fish of the class Chondrichthyes.

Keywords: blue shark, immunocytochemistry, *myc*, *p-53*, proliferating cell nuclear antigen, *ras*.

Introduction

Sharks, skates, rays and chimeras are gill breathing, jawed, finned, gas bladder-deficient fish with a calcified cartilaginous endoskeleton comprising the class Chondrichthyes. As Deslongchamps described a fibroma on the tail of a thornback skate, *Raja clavata* L., in 1853 (Thomas 1931), over 40 neoplasms have been discovered in sharks (Borucinska, Harshbarger & Bogicevic 2002). Despite this and the fact that tumours have been described from more species of free ranging wild-caught cartilaginous than bony fish (J. Harshbarger, personal communication; Borucinska *et al.* 2002), there is a prevailing hypothesis that cartilaginous fishes are resistant to cancer (Ballantyne 1997). Indeed several studies examined the anti-cancer potential of products derived from shark tissues, including cartilage (Felzenszwalb, Pelielo de Mattos, Bernardo-Filho & Caldeira-de-Araujo 1998; Miller, Anderson, Stark, Granick & Richardson 1998) and squalamine (Sills, Williams, Tyler, Epstein, Sipos, Davis, McLane, Pitchford,

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Cheshire, Gannon, Kinney, Chao, Donowitz, Laterra, Zasloff & Brem 1998). Although results from the above and other studies (Mathews 1992) did not support the postulated anti-cancer properties of shark tissues, the increased market demand for anti-cancer remedies and fish protein in general, led to drastic increases in shark harvesting worldwide. This, coupled with very slow reproductive rates in most shark species as compared with bony fish, led to collapses in several shark populations and seriously threatens the remaining shark stocks (Ballantyne 1997; NMSF (U.S. National Marine Fisheries Service) 1999).

This study was undertaken to detect molecular markers of neoplastic transformation in tissues from sharks and thus provide additional evidence of their susceptibility to neoplastic diseases. We used immunocytochemical procedures to visualize changes in the expression of proliferating cell nuclear antigen (PCNA), and to detect products of the *p-ras*, *c-myc* and *p-53* genes, all of which are well-established markers of neoplastic transformation in mammals and in bony fish (Baumann & Okihiro 2000). Previous investigations have documented cross-reactivity of tissue-specific antigens from bony fish and their mammalian counterparts as detected by immunocytochemistry (Bunton 1993). Subsequently, immunocytochemistry has been used in teleosts to diagnose tissue-specific antigens in tumours, and to study their alteration of cellular proliferation rates measured as increases in the expression of PCNA (Bunton 1994, 1995, 2000). Recent immunocytochemical studies of neoplasia in fish suggest that some of their tumours express detectable specific mutations in tumour suppressor genes and oncogenes, and can overexpress PCNA (Grizzle & Goodwin 1998; Baumann & Okihiro 2000). Thus, common genetic and epigenetic pathways of neoplastic transformation in cells from mammals and teleosts have been documented (Bunton 2000). Such data are not available for cartilaginous fish.

The immunocytochemical assay for PCNA has been used widely to assess the proliferation status of transformed cells in tumours from vertebrates. PCNA is a DNA polymerase delta auxiliary factor that is synthesized throughout the cell cycle, with the exception of the resting phase (Baumann & Okihiro 2000). PCNA has been detected in numerous cell types in mammalian tissue and recent studies have shown that aquatic species exhibit PCNA-positive staining in the primary gill filament, alimentary tract epithelium, and in cells from the testes of the medaka, *Oryzias latipes* (Temminck & Schlegel), guppy, *Poecilia reticulata* Peters, western mosquitofish, *Gambusia affinis* (Baird & Girard) (Ortego, Hawkins, Walker, Krol & Benson 1994) and the spiny dogfish shark, *Squalus acanthias* L. (McClusky 2005). Although the above studies documented that normally proliferating cells within labile tissues will exhibit PCNA positivity, an increased expression of this protein has been widely accepted as a marker of proliferation associated with neoplasia of normally stable cell populations (Ortego *et al.* 1994), and such increased expression has been documented in papillomas from fish (Bunton 2000).

The high degree of homology between the tumour suppressor gene *p-53* and oncogenes of the *myc* and *ras* families among bony fish and mammals has been shown in several studies (Caron de Fromentel, Pakdel, Chapus, Baney, May & Soussi 1992; Panno & McKeown 1995; Vincent, Jaunet, Galgani, Besselink & Koeman 1995; Krause, Rhodes & Van Beneden 1997; Peck-Miller, Myers, Collier & Stein 1998; Bhaskaran, May, Rand-Weaver & Tyler 1999; Franklin, Lee, Köhler & Chipman 2000; Vincent-Hubert 2000; Liu, Kullman, Bencic, Torten & Hinton 2003). Alterations in the expressions of the above genes in fish tumourigenesis have been widely documented in natural and experimental settings (Vincent, de Boer, Pfohl-Leszkowicz, Cherrel & Galgani 1998; Baumann & Okihiro 2000; Rotchell, Lee, Chipman & Ostrander 2001; Liu *et al.* 2003). For example, an increased expression of the nuclear oncogene *c-myc* has been shown in piscine biliary and hepatic neoplasms (Goodwin & Grizzle 1994; Carter, Ellington & Van Beneden 1996). The expression of the cytoplasmic membrane-associated oncogene products in the *ras* family has been studied in liver tumours, and to a limited degree in mesotheliomas, in fish (Wirgin, Currie & Garte 1989; McMahon, Huber, Moore, Stegeman & Wogan 1990; Chang, Mathews, Mangold, Marien, Hendricks & Bailey 1991; Goodwin & Grizzle 1994; Hendricks, Cheng, Shelton, Pereira & Bailey 1994; Orner, Mathews, Hendricks, Carpenter, Bailey & Williams 1995; Carter *et al.* 1996; Torten, Liu, Okihiro, Teh & Hinton 1996; Peck-Miller *et al.* 1998; Liu *et al.* 2003). Lastly, mutations and/or overexpression of the tumour suppressor gene *p-53* has been reported from hepatic tumours in bony fish (Goodwin & Grizzle 1994; Franklin *et al.* 2000).

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Materials and methods

Archival formalin-fixed, paraffin-embedded tissues from 10 free-ranging, adult male blue sharks, *Prionace glauca* (L.), were studied. All sharks were caught during summer months between 2000 and 2006 by recreational fishermen off the USA coast in the northwestern Atlantic. These sharks were part of studies on the occurrence of natural- or man-induced lesions in the tissues of wild-caught sharks. During each of the above summers, all blue sharks landed in our presence during fishing tournaments (between 50 and 80 fish each year) were necropsied on landing and all organs with macroscopic lesions were collected into 10% buffered elasmobranch formalin (Prieur, Fenstermacher & Guarino 1976). These organ samples were processed routinely for paraffin embedding and sectioned at 4–5 μm. The samples of hyperplastic, dysplastic or neoplastic lesions that were used in this study included two cases of gastric adenomatous polyps, a testicular mesothelioma, a fibropapillomatous epulis with elements of an ameloblastoma, a hepatic cholangiocarcinoma, a hepatocellular tumour and a mixed hepatic tumour, two cases of pericardial fibropapillomas, and proliferating mesothelium from six cases of proliferative peritonitis/pericarditis including a dysplastic mesothelium associated with granulomatous, parasitic enteritis. For negative control tissues, we used normal liver, glandular stomach, peritoneum, pericardium and gingiva from the above sharks in addition to normal gingiva and gastric fundic mucosa from two healthy sharks. An isotype negative control was also done for each slide examined. As positive controls, tumours from human cases routinely used by our diagnostic laboratory were used. The field data, tissues studied and references to published case reports describing the histopathology of some of the above lesions are provided in Table 1. The tumours from published reports were deposited in The Registry of Tumors in Lower Animals (hepatic carcinoma, testicular mesotheliona RTLA No. 7300) or in the archives of the Armed Forces Institute of Pathology (gingival tumour and gastric adenomatous polyp, AFIP No. 2859819). The remaining tumours will be described in a separate report.

Immunocytochemistry

Serial 4–5 μm thick sections were cut from paraffin blocks onto silane-coated glass slides. Slides were dried for 30 min at 60 °C, dewaxed in xylene and rehydrated through a graded alcohol series. Endogenous peroxidase activity was blocked with freshly prepared 3% hydrogen peroxide (aqueous; Sigma, St. Louis, MO, USA). Commercially available antibodies anti-PCNA (PC10, mouse monoclonal antibody derived against a recombinant mouse PCNA consisting of amino acids 112–121), anti-p-53 (PAb240, mouse monoclonal anti-human), anti-p-Ras (RAS10, mouse monoclonal antibody

Table 1 Tissues from blue sharks examined by immunocytochemistry

Shark number	Year collected	Body weight (kg)	Fork length (cm)	Structures examined	Reference[a]
1	2000	85	232	Gastric polyp	Borucinska & Bogicevic (2004)
2[b]	2000	55.8	262	Proliferative peritonitis	Borucinska *et al.* (2001)
3[b]	2000	133.2	269	Liver tumour, mesothelioma/testes, proliferative peritonitis	Borucinska *et al.* (2003)
4[c]	2000	99.3	252	Liver tumour, proliferative peritonitis, pericardial fibropapillomas	UD
5	2000	150.5	276	Gingival epulis, proliferative peritonitis, granulation tissue	Borucinska *et al.* (2004)
6[d]	2004	112.9	266	Liver tumour, proliferative peritonitis	UD
7	2005	101.6	257	Normal gingiva	UD
8[c]	2005	100.2	241	Pericardial fibropapillomas, proliferative peritonitis	UD
9	2006	145.6	302	Gastric polyp	UD
10	2006	133.3	268	Normal gastric mucosa	UD

UD, Unpublished data.
[a]Published case reports with histopathological description of tissues retrieved for the current study.
[b]Retained fishing hook with gastric perforation.
[c]Retained fishing hook, gastric perforation, hepatic laceration and herniation into the pericardial sac.
[d]Retained fishing hook with gastric perforation and hepatic laceration.

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derived against a recombinant human P-21 ras protein) and anti-c-myc (9E10, mouse monoclonal derived against a synthetic peptide equivalent to human c-myc amino acids 408–439; Calbiochem, San Diego, CA, USA), and anti-p-53 (clone DO-7, mouse monoclonal; DAKO, Carpinteria, CA, USA), were used. Pretreatment, antigen retrieval and final antibody concentration varied for each antibody and are described separately below.

For anti-PCNA, a modified method of McClusky (2005) was followed. Briefly, slides were incubated in ''Retrieve-all'' pH 8.0 (Signet Laboratories, Dedham, MA, USA) for 10 min in a steamer bath, followed by 10 min 'cool down' at room temperature (RT). Final antibody concentration was 0.001 $\mu g\ \mu L^{-1}$.

For anti-p-53, a modified method of Goodwin & Grizzle (1994) was followed. Briefly, pretreatment involved incubation in citrate buffer pH 6.0 for 40 min in a steamer bath, followed by 10 min 'cool down' at RT. Final antibody concentration was 0.001 $g\ \mu L^{-1}$.

For anti-p-ras and anti-c-myc, no pretreatment was required (Carter *et al.* 1996). Final antibody concentrations were 0.001 and 0.002 $g\ \mu L^{-1}$, respectively.

After pretreatment, the slides were washed twice, for 5 min each, at RT in diluted Cadenza buffer wash concentrate (Thermo Electron Corp., Waltham, MA, USA), which buffer was used for all the remaining washes. To block non-specific immunoassaying, the slides were incubated at RT for 5–10 min in Omnitags protein blocking agent (Thermo Electron Corp.). Protein blocking was followed by incubation of slides overnight at RT with 150 μL of optimally diluted primary antibody, optimally diluted mouse isotype antibody (Thermo Electron Corp.) or Cadenza buffer. After washing, biotinylated anti-rabbit and anti-mouse secondary agent (Thermo Electron Corp.) at 1:40 dilution was applied at RT for 30 min. The sections were then washed and incubated with Strept-Avidin peroxidase agent at 1:4 dilution for 20 min at RT, washed again, and developed at RT for 10 min with Nova red chromogen (Vector Nova Red Substrate Kit, Vector Laboratories, Burlingame, CA, USA). Sections were counterstained with Shandon Gill 2 haematoxylin (Thermo Electron Corp.), dehydrated, cleared and mounted with Permount (Fisher, Fairlawn, NJ, USA) and examined using brightfield microscopy. The proliferation index (PI) for PCNA stained slides from the neck region of gastric glands was calculated by counting positive nuclei in five fields at 40× magnification and recording the mean value.

Results

The *p-53* gene products were not detected in any tissues studied. The *c-myc* product, which is a nuclear oncoprotein, was detected only in one case of a hepatocellular tumour from shark No. 4. The positive signal was seen as brownish nuclear staining in a large number of neoplastic cells (Fig. 1). This positive hepatocellular tumour was composed of poorly differentiated polygonal to oval cells forming solid nests and cords and of proliferating bile ducts, both of which had positive staining; in addition some oval cell foci in adjacent normal liver stained positively. Oval cell foci could be proliferating omnipotential hepatocyte/biliary cell precursors or they may be compatible with proliferation of biliary epithelium described in toxic liver injury in bony fish (Hinton & Lauren 1990).

The *p-ras* product, which is a membrane-associated oncoprotein, was visualized as cytoplasmic brown-red granular to laminar staining. Positive cells were detected in neoplastic mesothelium from testicular mesothelioma in shark No. 3 (Fig. 2), in dysplastic mesothelium of the spiral intestine (shark No. 5), in hypertrophic mesothelial cells from three sharks with proliferative peritonitis (Nos 3, 4 and 6), in hypertrophic mesothelium and stromal

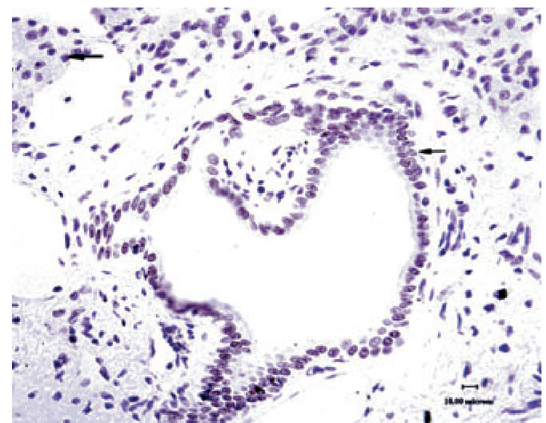

Figure 1 Poorly differentiated mixed tumour from liver of shark No. 4 stained with anti-myc antibody to detect expression of the nuclear oncoprotein c-myc. There are numerous positive nuclei (brown staining) in bile ducts (small arrow) and proliferating anaplastic cells from the liver tumour (large arrow). The connective tissue stroma is negative.

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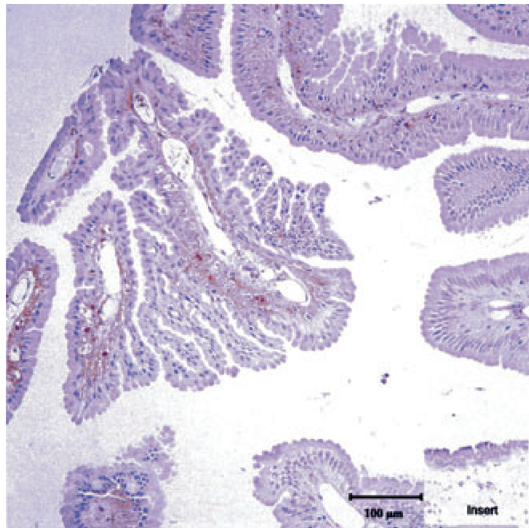

Figure 2 Testicular mesothelioma from shark No. 3 stained with the anti-ras antibody. There is cytoplasmic granular staining in the tall columnar hypertrophied tumour cells; insert shows the same magnification of similarly stained normal mesothelium from the non-affected portion of the testes of the same shark.

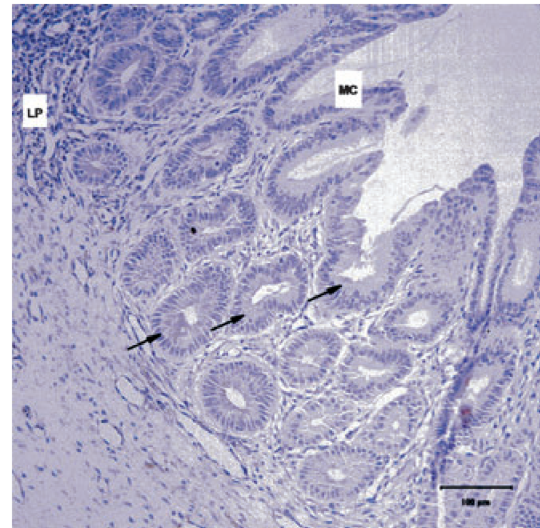

Figure 4 Gastric polyp from shark No. 1 stained with anti-ras antibody. Arrows point to crypts with intestinal metaplasia of glandular epithelium showing positive staining. The mucous cells lining the pits on the surface of the mucosa (MC) and the inflammatory cells in the lamina propria (LP) are negative.

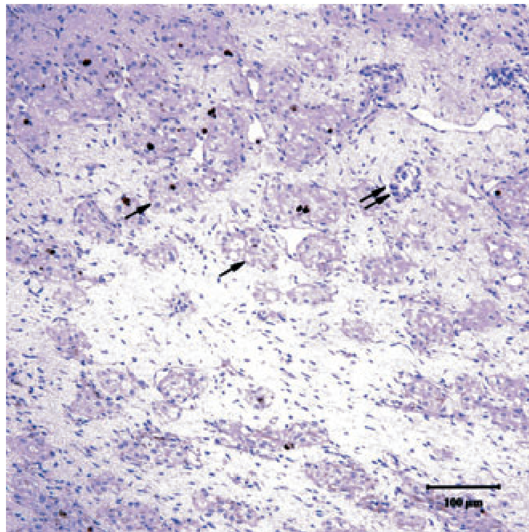

Figure 3 Poorly differentiated hepatocellular tumour from shark No. 4 stained with anti-ras antibody. Single arrows point to nests of positively staining neoplastic hepatocytes surrounded by abundant negative stroma; double arrows indicate a focus of negative inflammatory cells.

elements (endothelium and fibroblasts) from both sharks with epicardial fibropapillomas (Nos 4 and 8), in oval cell foci and in the hepatocellular tumour in shark No. 4 (Fig. 3), in a mixed liver tumour in shark No. 6, in foci of intestinal metaplasia in a gastric polyp from shark No. 1 (Fig. 4), in foci of stratified squamous metaplasia in a gastric polyp from shark No. 9 and both in dysplastic mesothelium and in gingival epithelium in epulis from shark No. 5.

The PCNA signal, seen as brownish nuclear staining, was detected in several normal and abnormal (hyperplastic, dysplastic and neoplastic) tissues in this study. The normal structures included germinal centres of secondary lymphoid follicles associated with gastric mucosa (shark No. 2), haematopoietic cells within the epigonal organ (shark No. 3; Fig. 5), the lower 1/3 layers of keratinocytes of gingival epithelium (sharks Nos 5 and 7), the neck /proliferative zone of normal gastric glands (sharks No. 1 and 9, PI 55), and the intestinal crypt epithelium in the spiral intestine (shark No. 5). The abnormal tissues showing positive PCNA staining included testicular mesothelioma (shark No. 3; Fig. 5), mesothelium and stromal elements (fibroblasts and endothelium) in pericardial fibropapillomas (sharks Nos 4 and 8), hypertrophied or dysplastic mesothelium associated with gastritis, enteritis or hepatitis/hepatic tumours (sharks Nos 2, 3, 4, 5, 6 and 8), cholangiocarcinoma cells (shark No. 3; Fig. 6), hepatocellular tumour cells (sharks Nos 2 and 4), proliferating bile ducts and stromal elements (fibroblasts and endothelium) in a mixed hepatic tumour (shark No. 6), fibroblasts and endothelium in granulation tissue in

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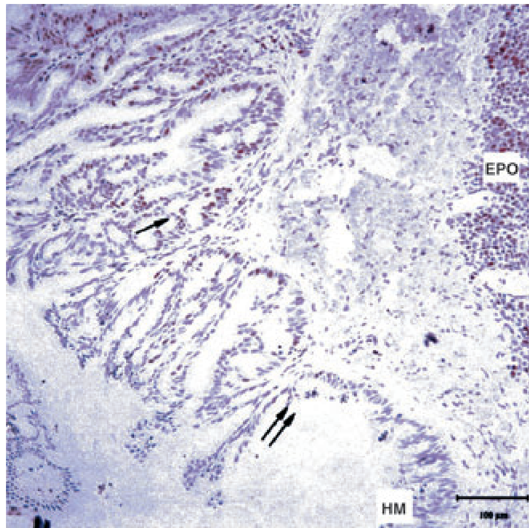

Figure 5 Testicular mesothelioma from shark No. 3 stained with the anti-proliferating cell nuclear antigen antibody. There is strong nuclear staining in the mesothelioma (single arrow) which ends abruptly (double arrow), and no nuclear staining is present in the adjacent hypertrophied mesothelium (HM). The haematopoietic cells within the epigonal organ also have positive nuclei (EPO).

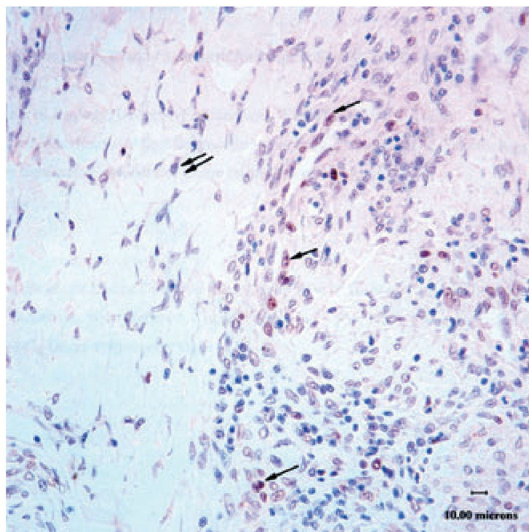

Figure 6 Cholangiocarcinoma from shark No. 3 stained with the anti-proliferating cell nuclear antigen antibody. There is strong nuclear staining in the tumour cells (single arrows) but no staining in the stromal elements (double arrows).

the lamina propria of the spiral intestine with mesothelial dysplasia (shark No. 5), neck zone of gastric glands (PI 70) and gastric glands with intestinal metaplasia (PI 300) in a gastric polyp (shark No. 1). A summary of all immunocytochemistry results is provided in Table 2.

Discussion

This study is the first literature report on the detection of molecular markers of neoplastic transformation in tissues from cartilaginous fish. We have documented positive p-ras, c-myc and PCNA signals in several tissues that had histological characteristics of neoplastic lesions. In addition, we detected similar positive signals in some lesions of hyperplasia, dysplasia and metaplasia, which points to the possibility of early transformation events in the above tissues. This is a significant finding because it might indicate the progression of some of these lesions to neoplasia. As was expected based on results obtained by previous investigators (McClusky 2005), we detected a strong PCNA signal in normal, labile tissues with physiologically high cell-turnover rates including intestinal crypts, gingival mucosa and lymphopoietic and haematopoietic structures. Interestingly, the neck zones of gastric glands were positive adjacent to a gastric polyp, but negative in a shark with a normal gastric mucosa (shark No. 10). The level of mitotic activity is usually very low in a normal gastric mucosa in sharks (J. Borucinska, unpublished observations), so the PCNA positivity adjacent to the gastric polyps might indicate an increased turnover rate in an inflamed mucosa which is very frequently considered to be involved in the pathogenesis of so-called inflammatory polyps. Further studies are needed to elucidate the reasons for the absence of p-53 staining in our tissues, but immunocytochemical results could be influenced by prolonged formalin fixation, lack of cross-reactivity between the mammalian and piscine protein, or other unknown factors. More tumours from sharks and bony fish have to be studied to confirm and expand our results, including the detection of the abnormal p-53 protein in these lesions. Because all antibodies used in this study were generated in mice using mammalian or synthetic antigens, the signal produced in shark tissues was quite weak. It would be worthwhile to repeat our studies with piscine reagents that are currently not available.

The results from this study and the case reports of tumours in sharks from our and other laboratories (Prieur *et al.* 1976; Berzins & Hovland 1999; Borucinska *et al.* 2002; Borucinska & Bogicevic 2004; Borucinska, Harshbarger, Reimschuessel &

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Table 2 Summary of immunocytochemistry results for normal, hyperplastic, dysplastic and neoplastic tissues from blue sharks stained with the anti-PCNA, anti-ras and anti-myc antibodies

Structures[a]	PCNA	p-ras	c-myc
Neck zone of gastric glands adjacent to polyp (PI[b] 55)	+	+	–
Intestinal metaplasia within gastric polyp[a] (PI 300)	+	+	–
Neck zone of gastric glands in gastric polyp[a] (PI 77)	+	+	–
Neck zone of gastric glands with no mucosal lesions	–	–	–
Squamous metaplasia of gastric mucosa within gastric polyp[a]	+	–	–
Normal mesothelium	–	–	–
Hyperplastic and hypertrophic mesothelium	+/–	+	–
Dysplastic mesothelium	+	+	–
Mesothelioma[a]	+	+	–
Haematopoietic cells within epigonal organ	+	–	–
Lymphoblasts in lymphofollicular structures	+	–	–
Fibroblasts/endothelium in granulation tissue	+	–	–
Normal hepatocytes	–	–	–
Hepatocellular carcinoma[a]	+	+	–
Normal bile ducts	–	–	–
Hyperplastic/hypertrophic bile ducts	+	–	+
Fibroblasts and endothelium within fibroplasia in cholangiocarcinoma[a]	+	+	–
Cholangiocarcinoma[a]	+	–	–
Hepatocellular tumour[a]	+	+	+
Oval cell foci within liver with hepatocellular tumour	nd	+	+
Mixed liver tumour[a]	+	+	–
Fibroblasts and endothelium within epicardial fibropapillomas[a]	+	+	–
Mesothelium in epicardial fibropapillomas[a]	+	+	–
Keratinocytes in normal gingival mucosa	+	–	–
Keratinocytes in gingival papilloma[a]	+	+	–
Intestinal crypts in the spiral intestine	+	–	–

PCNA, proliferating cell nuclear antigen; PI, proliferative index; nd, not done.
[a]Indicates neoplastic lesions.

Bogicevic 2004) provide further evidence to invalidate the hypothesis that sharks have a higher resistance to tumours than other vertebrates. Ballantyne (1997) cited the Registry of Tumours in Lower Vertebrates as his source for the data supporting the hypothesis of low prevalence of tumours in sharks as compared with other fish data, which since then have been expanded to refute this very hypothesis (Borucinska *et al.* 2002, 2004). There are several important reasons for the low numbers of cancer and, for that matter any diseases, reported from sharks (Grizzle & Goodwin 1998). First, there are very small numbers of sharks held in captivity or used in research, and no sharks are used in aquaculture, all of which were historically a source of data on neoplasia in teleost fish. In addition, contrary to Ballantyne's (1997) statement that thousands of sharks have been examined, our own research indicates that the actual number of sharks studied by pathologists is very small. Adding to this problem is the fact that many tumours in sharks, for example hepatic tumours, can be detected only by histological examination (J. Borucinska, unpublished observations), and thus many tumours can be missed by solely visual inspection of sharks. Lastly, the sharks collected in the wild represent the actively hunting, and thus healthy population, and diseased specimens enter the ocean's food chain before they can be examined.

The presence of tumours in populations of wild fish has been used for a long time as a bioindicator of fish health and ecosystem health (Baumann 1992). The high trophic level of most shark species coupled with their cosmopolitan migrations and longevity makes them excellent sentinel species of global marine contamination with potentially carcinogenic compounds. In addition, because most of the detoxifying enzyme systems in the liver of sharks are compatible with higher vertebrates (Ballantyne 1997), disease data from sharks can be of value in assessing the impact of low level, chronic exposure to environmental anthropogenic toxins on animal and human health. A similar application has been recently postulated for the study of cancer in marine mammals (Newman & Smith 2006).

The primary goal of our research was to deepen our understanding of carcinogenesis by showing shared molecular characteristics of neoplastic cells in sharks and in higher vertebrates. We would also hope that this paper will contribute to the recently

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recognized need (Ostrander, Cheng, Wolf & Wolfe 2004) to protect and study sharks as indispensable members of the marine ecosystem, and to diminish their slaughter to harvest the scientifically not endorsed "anti-cancer" remedies from their tissues.

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References

Ballantyne J.S. (1997) Jaws: the inside story. The metabolism of elasmobranch fishes. *Comparative Biochemistry and Physiology* **118B,** 703–742.

Baumann P.C. (1992) The use of tumors in wild populations of fish to assess ecosystem health. *Journal of Aquatic Ecosystem Health* **1,** 135–146.

Baumann P.C. & Okihiro M.S. (2000) Cancer. In: *The Laboratory Fish* (ed. by G.K. Ostrander), pp. 591–616. Academic Press, London.

Berzins I.K. & Hovland E. (1999) Shark tumor: an oxymoron. In: *Proceedings of the Annual Meeting, International Association for Aquatic Animal Medicine* (ed. by K.D. Arkush), pp. 3–4. International Association of Aquatic Animal Medicine, Washington State University, College of Veterinary Medicine.

Bhaskaran A., May D., Rand-Weaver M. & Tyler C.R. (1999) Fish p-53 as a possible biomarker for genotoxins in the aquatic environment. *Environmental Molecular Mutagenesis* **33,** 177–184.

Borucinska J.D. & Bogicevic T. (2004) Gastric polyp in a wild-caught blue shark. *Journal of Aquatic Animal Health* **16,** 39–44.

Borucinska J.D., Harshbarger J.C. & Bogicevic T. (2002) Hepatic cholangiocarcinoma and testicular mesothelioma in a wild-caught blue shark, *Prionace glauca* (L.). *Journal of Fish Diseases* **25,** 1–7.

Borucinska J.D., Martin J. & Skomal G. (2001) Peritonitis and pericarditis associated with gastric perforation by a retained fishing hook in a blue shark (*Prionace glauca*). *Journal of Aquatic Animal Health* **13,** 347–354.

Borucinska J.D., Harshbarger J.C. & Bogicevic T. (2003) Hepatic cholangiocarcinoma and testicular mesothelioma in a wild-caught blue shark (*Prionace glauca*) (Linnaeus, 1758). *Journal of Fish Diseases* **26,** 43–49.

Borucinska J.D., Harshbarger J.C., Reimschuessel R. & Bogicevic T. (2004) Gingival neoplasms in a captive sand tiger shark, *Carcharias taurus* (Rafinesque), and a wild-caught blue shark, *Prionace glauca* (L.). *Journal of Fish Diseases* **27,** 185–191.

Bunton T.E. (1993) The immunocytochemistry of cytokeratin in fish tissues. *Veterinary Pathology* **30,** 418–425.

Bunton T.E. (1994) Intermediate filament reactivity in hyperplastic and neoplastic lesions from medaka (*Oryzias latipes*). *Experimental Toxicologic Pathology* **46,** 389–396.

Bunton T.E. (1995) Expression of actin and desmin in experimentally induced hepatic lesions and neoplasms from medaka (*Oryzias latipes*). *Carcinogenesis* **16,** 1059–1063.

Bunton T.E. (2000) Brown bullhead (*Ameiurus nebulosus*) skin carcinogenesis. *Experimental Toxicologic Pathology* **52,** 209–220.

Caron de Fromentel C., Pakdel F., Chapus A., Baney C., May P. & Soussi T. (1992) Rainbow trout *p-53*: cDNA cloning and biochemical characterization. *Gene* **112,** 241–245.

Carter C.A., Ellington W.W. & Van Beneden R.J. (1996) Confocal laser scanning microscopy of oncogene localization in rainbow trout cell lines derived from normal and tumor tissue. *Toxicologic Pathology* **24,** 339–345.

Chang Y., Mathews C., Mangold K., Marien K., Hendricks J. & Bailey G. (1991) Analysis of *ras* gene mutations in rainbow trout liver tumors initiated by aflatoxin B1. *Molecular Carcinogenesis* **4,** 112–119.

Felzenszwalb I., Pelielo de Mattos J.C., Bernardo-Filho M. & Caldeira-de-Araujo A. (1998) Shark cartilage-containing preparation: protection against reactive oxygen species. *Food Chemistry and Toxicology* **12,** 1079–1084.

Franklin T.M., Lee J., Köhler A. & Chipman J.K. (2000) Analysis of mutations in the *p-53* tumor suppressor gene and Ki- and Ha-*ras* proto-oncogenes in hepatic tumors of European flounder (*Platichthys flesus*). *Marine Environmental Research* **50,** 251–255.

Goodwin A.E. & Grizzle J.M. (1994) Oncogene expression in hepatic and biliary neoplasm of the fish *Rivulus ocellatus marmoratus*: correlation with histologic changes. *Carcinogenesis* **15,** 1993–2002.

Grizzle J.M. & Goodwin A.E. (1998) Neoplasms and related lesions. In: *Fish Diseases and Disorders, Vol.2, Non-Infectious Disorders* (ed. by J.F. Leatherland & P.T.K. Woo), pp. 37–104. CAB International, Wallingford, Oxon, UK.

Hendricks J.D., Cheng R., Shelton D.W., Pereira C.B. & Bailey G.S. (1994) Dose-dependent carcinogenicity and frequent Ki-*ras* proto-oncogene activation by dietary *N*-nitrosodiethylamine in rainbow trout. *Fundamental and Applied Toxicology* **23,** 53–62.

Hinton D.E. & Lauren D.J. (1990) Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: *Biomarker of Environmental Contamination* (ed. by J.F. McCarthy & L.R. Shugart), pp. 17–58. Lewis Publishers, Chelsea, MI.

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Krause M.K., Rhodes l.D. & Van Beneden R.J. (1997) Cloning of the *p-53* tumor suppressor gene from Japanese medaka (*Oryzias latipes*) and evaluation of mutational hotspots in MNNG-exposed fish. *Gene* **189,** 101–106.

Liu Z., Kullman S.W., Bencic D.C., Torten M. & Hinton D.E. (2003) *Ras* oncogene mutations in diethylnitrosamine-induced hepatic tumors in medaka (*Oryzias latipes*), a teleost fish. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* **539,** 43–53.

Mathews J. (1992) Sharks still intrigue cancer researchers. *Journal of the National Cancer Institute* **84,** 1000–1002.

McClusky L.M. (2005) Stage and season effects on cell cycle and apoptotic activities of germ cells and Sertoli cells during spermatogenesis in the spiny dogfish (*Squalus acanthias*). *Reproduction* **129,** 89–102.

McMahon G., Huber L.J., Moore M.J., Stegeman J.J. & Wogan G.N. (1990) Mutations in c-Ki-*ras* oncogenes in diseased livers of winter flounder from Boston Harbor. *Proceedings of the National Academy of Science* **87,** 841–845.

Miller D.R., Anderson G.T., Stark J.J., Granick J.L. & Richardson D. (1998) Phase I/II trial of the safety and efficacy of shark cartilage in the treatment of advanced cancer. *Journal of Clinical Oncology* **16,** 3649–3655.

Newman S.J. & Smith S.A. (2006) Marine mammal neoplasia: a review. *Veterinary Pathology* **43,** 865–880.

NMSF (U.S. National Marine Fisheries Service) (1999) *Fishery Management Plan for Atlantic Tunas, Swordfish, and Sharks, Vol. I.* U.S. Department of Commerce, Washington D.C.

Orner G.A., Mathews C., Hendricks J.D., Carpenter H.M., Bailey G.S. & Williams D.E. (1995) Dehydroepiandrosterone is a complete hepatocarcinogen and potent tumor promoter in the absence of peroxisome proliferation in rainbow trout. *Carcinogenesis* **16,** 2893–2898.

Ortego L.S., Hawkins W.E., Walker W.W., Krol R.M. & Benson W.H. (1994) Detection of proliferating cell nuclear antigen in tissues of three small fish species. *Biotechnic & Histochemistry* **69,** 317–323.

Ostrander G.K., Cheng K.C., Wolf J.C. & Wolfe M.J. (2004) Shark cartilage, cancer and the growing threat of pseudoscience. *Cancer Research* **64,** 8485–8491.

Panno J.P. & McKeown B.A. (1995) Cloning and expression of a myc family member from the pituitary gland of the rainbow trout, *Oncorhynchus mykiss. Biochimica et Biophysica Acta* **1264,** 7–11.

Peck-Miller K.A., Myers M., Collier T.K. & Stein J.E. (1998) Complete c-DNA sequence of the Ki-*ras* proto-oncogene in the liver of wild English sole (*Pleuronectes vetulus*) and mutation analysis of hepatic neoplasms and other toxicopathic liver lesions. *Molecular Carcinogenesis* **23,** 207–216.

Prieur D.J., Fenstermacher J.D. & Guarino A.M. (1976) A choroid plexus papilloma in an elasmobranch (*Squalus acanthias*). *Journal of the National Cancer Institute* **56,** 1207–1209.

Rotchell J.M., Lee J., Chipman J.K. & Ostrander G.K. (2001) Structure, expression and activation of fish *ras* genes. *Aquatic Toxicology* **55,** 1–21.

Sills A.K., Williams J.I., Tyler B.M., Epstein D.S., Sipos E.P., Davis J.D., McLane M.P., Pitchford S., Cheshire K., Gannon F.H., Kinney W.A., Chao T.L., Donowitz M., Laterra J., Zasloff M. & Brem H. (1998) Squalamine inhibits angiogenesis and solid tumor growth *in vivo* and perturbs embryonic vasculature. *Cancer Research* **58,** 2784–2792.

Thomas L. (1931) Les tumeurs des poissons (etude anatomique et pathogenique). *Bulletin Association Francais Etude Cancer* **20,** 703–760.

Torten N., Liu Z., Okihiro M.S., Teh S.J. & Hinton D.E. (1996) Induction of *ras* oncogene mutations and hepatocarcinogenesis in medaka (*Oryzias latipes*) exposed to diethylnitrosamine. *Marine Environmental Research* **42,** 93–98.

Vincent F., Jaunet S., Galgani F., Besselink H. & Koeman J. (1995) Expression of *ras* gene in flounder (*Platichthys flesus*) and red mullet (*Mullus barbatus*). *Biochemical and Biophysical Research Communications* **215,** 659–665.

Vincent F., de Boer J., Pfohl-Leszkowicz A., Cherrel Y. & Galgani F. (1998) Two cases of *ras* mutations associated with liver hyperplasia in dragonets (*Callionymus lyra*) exposed to polychlorinated biphenyls and polycyclic aromatic hydrocarbons. *Molecular Carcinogenesis* **21,** 121–127.

Vincent-Hubert F. (2000) cDNA cloning and expression of two Ki-ras genes in the flounder, *Platichthys flesus*, and analysis of hepatic neoplasms. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* **126,** 17–27.

Wirgin I., Currie D. & Garte S.J. (1989) Activation of the K-oncogene in liver tumors of Hudson River tomcod. *Carcinogenesis* **10,** 2311–2315.

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