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Cancer research using the medaka, *Oryzias latipes*, over 21 years*

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Prologue

It was 21 years ago that medaka fish were first used for cancer research by Ishikawa, one of the present authors, in 1975. Since that time, the medaka has become established in the main stream of cancer research using fish, both within Japan and in the United States of America. At the time of writing, out of 36 papers dealing with neoplasia in the medaka were published during the past 20 years, 19 were from Japan and the remaining 17 were all from the USA. A comparison of the two countries' outputs in this area over time is shown in Fig. 1. In Japan, five or six papers documenting findings in cancer research using medaka were published every four years between 1975 and 1989, with a decline thereafter from 1990 to 1994. In the USA, the first paper dealing with medaka appeared only in 1984 (Klaunig *et al.*, 1984) but the subsequent rapid rise in interest is now reflected in an output three times that of Japan.

In fact, the first cancer research using small aquarium fish was the laboratory work with the zebrafish, *Branchydanio rerio*, conducted by Stanton in 1965 in the USA. The medaka is in no way inferior to the zebrafish which has received a great deal of attention (Mullins and Nüsslein-Volhard, 1993; Driever *et al.*, 1994), and its advantages for many purposes deserve emphasis and note by the younger generation of research workers. Thus, it is timely that this special edition be published to follow on from earlier reviews of the subject (Briggs and Egami, 1959; Ishikawa *et al.*, 1975; Ishikawa *et al.*, 1984) by pointing out that: 1) The medaka, and especially the orange-red

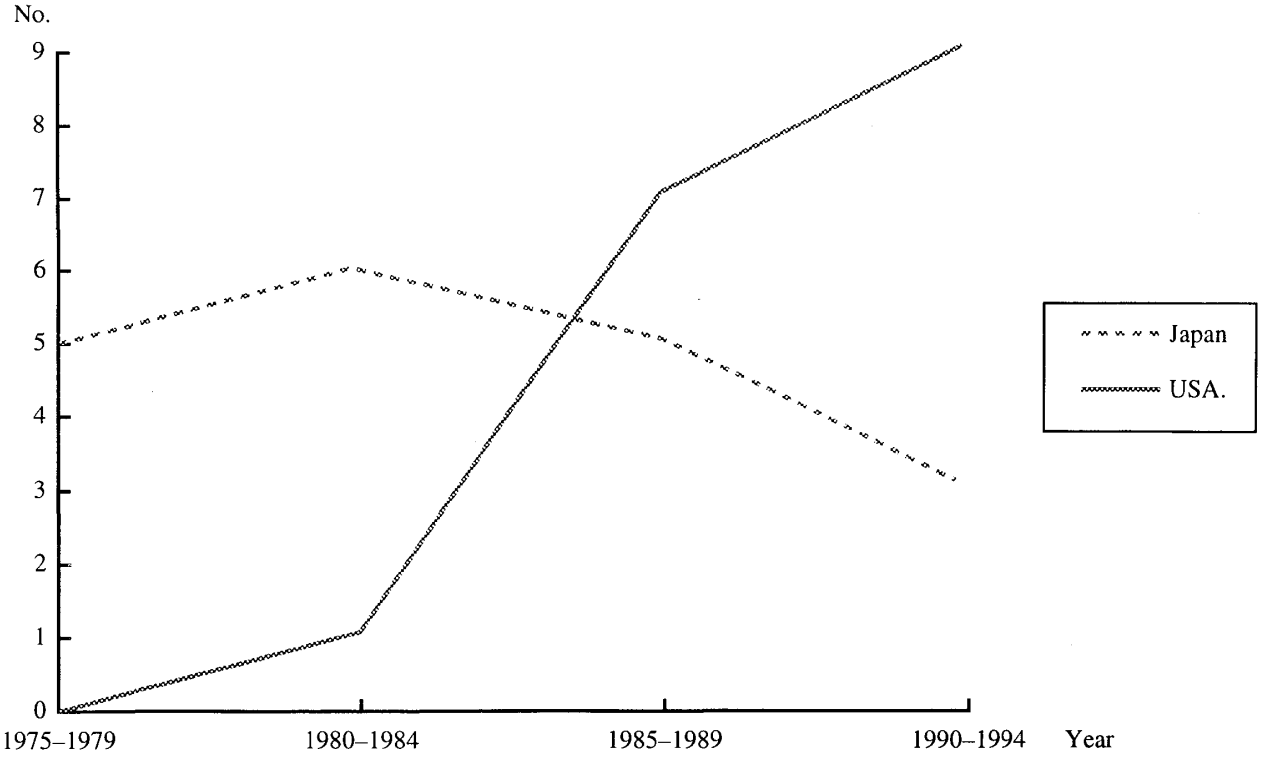

Fig. 1. Number of cancer research papers published featuring use of medaka.

* This paper contributes to the Special Issue "Development of Medaka Biology in Japan."

variety (himedaka), is the most common aquarium fish other than goldfish and ornamental carp in Japan; 2) Large numbers of individuals can be maintained in a limited space because of their small size; 3) The medaka is easy to breed under favorable laboratory conditions with males and females being easily distinguishable by the shape of their anal fins; 4) This fish can adapt to a wide range of temperature (5-30°C).

Chemical induction of tumors in the medaka

Since the first successful induction of liver tumors (Ishikawa *et al.*, 1975), experimental hepatocarcinogenesis in the medaka has been achieved with various carcinogens using different methods from 1975 to 1984 by Japanese researchers. In the initial study, addition of diethylnitrosamine (DEN) to the aquarium water at levels of 15-135 ppm for eight weeks resulted in hepatocellular carcinoma development after 13 weeks in 21 of 32 fish. Examination of the relation between the DEN exposure period and the incidence of tumors by Ishikawa and Takayama in 1979, revealed that a large number of medaka treated with 45 ppm DEN and maintained at 25°C had tumors after only 6-8 weeks (Table 1).

Table 1. The relation between the period of exposure to 45 ppm DEN and the incidence of liver tumors in himedaka. Essentially simplified from Ishikawa and Takayama (1979).

Effective no. of fish	Exposure period (wks.)	No. of tumor-bearing fish (%)
16	8	16 (100)
86	6	40 (47)
98	4	39 (40)
92	2	7 (0.8)
89	1	0 (0)

Kyono and Egami (1977) studied the effect of temperature during and after DEN treatment and found the tumor incidence in medakas treated with DEN at 8°C to be very low in comparison with the value of 25°C. Similar results concerning the effect of temperature in DEN-induced hepatocarcinogenesis in the medakas were also obtained in other studies, (Kyono, 1978; Ishikawa and Takayama, 1979; Egami *et al.*, 1981). Egami *et al.* (1981) further reported that partial hepatectomy enhances liver tumor formation in the medaka, as it is well known to do in rodents. Hatanaka *et al.* (1982) succeeded in inducing hepatocellular carcinomas in the medaka with aflatoxins B_1 and G_1, sterigmatocystin, ortho-aminoazotoluene, methylazoxymethanol (MAM) acetate, and DEN treatment for 24 weeks, indicating a general sensitivity, again equivalent to that observed for rats and mice.

Aoki and Matsudaira (1977, 1981) concentrated attention on induction of hepatocellular carcinomas in the medaka after treatment with MAM acetate in aqueous solution, surprisingly, finding liver tumors, hepatocellular carcinomas and cholangiomas in 60 to 100% of fish 60 to 90 days after exposure to 2.0 to 3.0 ppm for only 24 hours (Aoki and Matsudaira, 1977). They further described production of tumors within the same time span by exposure to 10 ppm MAM acetate for as little as 1 hour (Aoki and Matsudaira, 1981). MAM-induced hepatocarcinogenesis in the medakas has subsequently been investigated in detail by both Japanese and American researchers (Harada *et al.*, 1988; Hawkins *et al.*, 1988a). Harada *et al.* (1988) examined the effect of MAM acetate on carcinogenesis in the medaka at low doses (0.1, 0.3 ppm), whereas Hawkins *et al.* (1988a) used a high dose (50 ppm) for the study of tumorigenesis. All the medakas were supplied from the breeding stocks of their own institutes (Aoki and Matsudaira, 1977, 1981; Harada *et al.*, 1988; Hawkins *et al.*, 1988a). From the data in Table 2, it would appear that there are some differences in susceptibility to MAM acetate among their colonies. All medakas used by Aoki and Matsudaira (1977, 1981) died within 9 days after 2-hr treatment with 50 ppm MAM acetate (unpublished data by Aoki). The same dose was used by Hawkins *et al.* (1988a), but their medakas survived in spite of being maintained at nearly the same water temperature (Table 2).

Mitani and Egami (1980) were able to subculture a liver tumor induced by DEN in a medaka, *in vitro* at 26°C for 18 passages over a period of more than five months. However, they could not establish cultures from normal liver cells. Generally speaking, the transplantation of fish tumors is difficult because of the lack of immunocompatibility. Hyodo-Taguchi and Matsudaira (1984) overcame this problem by producing inbred strains of medaka through repeated sister-brother matings for more than 30 generations from 1974. Thereby they achieved successful transplantation of melanomas produced by 20-100 ppm N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)

Table 2. Cancer research with the himedaka using MAM acetate.

Reference	Dose (ppm)	Exposure time	Water temperature	Observation time after treatment (days)	No. of fish examined	No. of fish with tumors (%)
Hawkins *et al.*, 1988a	50	2 hrs	26 ± 1°C	56	343	153 (45)
Aoki and Matsudaira, 1981	10	1 hr	25 ± 1°C	90	15	10 (67)
	8	1 hr	25 ± 1°C	90	18	9 (50)
	3	1 day	25 ± 1°C	90	3	3 (100)
	2	1 day	25 ± 1°C	90	12	9 (75)
	0.5	10 days	25 ± 1°C	90	12	10 (83)
Hatanaka *et al.*, 1982	0.3	63 days	25 ± 3°C	63	13	1 (8)
	0.3	63 days	25 ± 3°C	63	13	3 (23)
Harada *et al.*, 1988	0.3	3 days	25 ± 3°C	126	56	3 (5)
	0.3	3 days	25 ± 3°C	126	56	3 (5)
	0.1	14 days	25 ± 3°C	168	56	0 (0)
Aoki and Matsudaira, 1981	0.1	120 days	25 ± 1°C	90	10	1 (10)

exposure for 2 hrs, to syngenetic hosts. Melanomas developed only in the inbred strain HB3LC established from wild medaka. In contrast, almost no melanomas arose in the inbred strain HO4C derived from himedaka, although this strain proved sensitive to the acute toxicity of MNNG (Hyodo-Taguchi and Matsudaira, 1984; Hyodo-Taguchi and Egami, 1985).

Early stage of carcinogenesis in the medaka

Normal liver tissue in the medaka differs considerably from that of mammals, consisting of sheet-like arrangements of parenchymal cells with interlacing sinusoids and a few bile ducts. Histologically, however, the liver tumors in medaka do not generally differ to any great extent from equivalent mammalian tumors (Ishikawa *et al.*, 1975; Ishikawa and Takayama, 1979; Harada *et al.*, 1988). Nakazawa *et al.* (1985) reported enzyme-altered foci development after four to six weeks chronic treatment with 50 ppm DEN. ATPase and glucose-6-phosphatase activities were decreased in most foci, but increased in some others.

In fish, DNA damage induced by ultraviolet radiation or carcinogens can be repaired by mechanisms such as excision, photoreactivation, and O^6-methylguanine DNA methyltransferase (O^6-MT)-enzyme action. Unscheduled DNA synthesis (UDS) has been demonstrated by autoradiography in medaka ganglion cells in response to a number of chemical carcinogens, including methyl methanesulfonate, methylnitrosourea, MNNG, N-hydroxy-2-fluorenylacetamide, 4-nitroquinoline 1-oxide, 4-hydroxyaminoquinoline 1-oxide, dimethylnitrosamine, DEN, benzo[a] pyrene, and aflatoxin B_1 (Ishikawa *et al.*, 1984). However, no UDS was detected in liver or intestinal cells. O^6-MT is considered to play an important role in the repair of alkylating carcinogen-induced lesions in mammalian species. The level of O^6-MT activity in medaka is almost the same as in the mouse (Nakatsuru *et al.*, 1987), but when fish were exposed continuously to MAM acetate at levels of 0.1, 0.15, and 0.3 ppm in their water, O^6-MT activity was markedly reduced from days one to seven with a slight increase thereafter. The O^6-MT activity also decreases with advancing age (Aoki *et al.*, 1993).

Spontaneous tumor in the medaka

Hawkins *et al.* (1988a) described the incidence of spontaneous tumors in young medakas to be almost zero, only one liver tumor and one lymphoma being observed in several thousand fish.

The incidence of spontaneous liver tumors was surveyed in 1- to 5-year-old medakas by Prince Masahito *et al.*, 1989 (Table 3). In 1-year-old fish, no neoplastic lesions were found and in the following two-years the incidence was relatively low. Liver tumors became more common with advancing age, reaching an incidence of 7.1% in 5-year-old (near-maximal-age) female medakas. These medaka had been kept in the ponds at the Division of Biology, National Institute of Radiological Sciences, Chiba-City from 1970 (Hyodo-Taguchi, 1990).

Four squamous cell carcinomas, five melanomas and four lymphomas were found in 13 medakas collected at three different sites in the medaka. Two of the melanomas and all the lym-

Table 3. Age dependence of the spontaneous liver tumor incidence in himedaka. Data from Prince Masahito et al., 1989. HCC = Hepatocellular carcinoma.

Age (Year)	No. of fish examined	Sex	No. of fish with liver tumors (%)	Diagnosis (No. of cases)	
				Adenoma	HCC
1	57	M	0 (0)	0	0
	67	F	0 (0)	0	0
2	108	M	2 (1.9)	2	0
	115	F	2 (1.7)	1	1
3	103	M	0 (0)	0	0
	151	F	2 (1.3)	0	2
4	138	M	2 (1.4)	2	0
	140	F	8 (5.7)	4	4
5	26	M	1 (3.8)	1	0
	56	F	4 (7.1)	3	2
Total	961	—	21 (2.2)	13	9

phomas showed systemic invasion (Prince Masahito *et al.*, 1989). Three out of four lymphomas were observed in the opeculum. Harada *et al.* (1990) also mentioned a lymphoma occurring in the kidney of one animal. Although the differentiation between T and B lymphocytes is not strictly defined in fish immune systems (Irwin and Kaattari, 1986), the former may originate from the thymus and the latter from the lymphatic organ in the kidney. A single olfactory neuroepithelioma (Torikata *et al.*, 1989) and one ovarian dysgerminoma (Harada *et al.*, 1991) have also been described as spontaneous tumors in the medaka.

Epilogue

Although, there have been 17 papers dealing with cancer research using medakas from laboratories in the USA over the past 21 years, the purpose of the present review was to survey the history of Japanese cancer research with this species. Therefore, these papers from the USA, including many excellent examples, are here only listed in Additional References.

The medaka has made contributions to Japanese biological study for longer than just the 21 years of use for cancer research. In fact, work on medaka genetics was already being conducted by Aida in 1921. Hyodo-Taguchi and her collaborators have now produced inbred medakas (1984, 1985). Ozato *et al.* (1986) have shown that transgenic methods can also be applied to the medaka. Very recently, Wada *et al.* (1995) have even made a start at producing a genetic linkage map. These new methods would seem to be also profitable for cancer researchers using the medaka. For any readers who want to pursue cancer research using medaka, the authors would strongly recommend study of the general biology of this fascinating animal. We sincerely hope that this edition may be of some assistance in providing a basis for future research.

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