ONCOLOGY REPORTS 16: 249-255, 2006

SPANDIDOS PUBLICATIONS

Expression of type I interferon receptor as a predictor of clinical response to interferon-α therapy of gastrointestinal cancers

HIDEO OTA[1], HIROAKI NAGANO[1], YUICHIRO DOKI[1], MITSUGU SEKIMOTO[1], MOTOI KONDO[1], HIROSHI WADA[1], MASATO NAKAMURA[1], TAKEHIRO NODA[1], BAZARRAGCHAA DAMDINSUREN[1], SHIGERU MARUBASHI[1], ATSUSHI MIYAMOTO[1], YUTAKA TAKEDA[1], KEIZO DONO[1], KOJI UMESHITA[1], SHOJI NAKAMORI[1], KENICHI WAKASA[2], MASATO SAKON[1] and MORITO MONDEN[1]

[1]Department of Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka E-2, Suita, Osaka 565-0871, [2]Department of Pathology, Osaka City University Hospital, 1-5-7 Asahi-cho, Abeno-ku, Osaka 545-0051, Japan

Received February 20, 2006; Accepted March 13, 2006

Abstract. Interferon (IFN) is used in the treatment of many malignancies and viral disorders. We recently reported a significant correlation between the efficacy of IFN-α combined with chemotherapy in the treatment of advanced hepatocellular carcinoma (HCC) and IFN-α/type I IFN receptor (IFNAR2) expression. It is possible that the expression of IFNAR2 in gastrointestinal cancerous tissue, apart from HCC, may predict the efficacy of IFN-α combination therapy. We investigated the expression of IFNAR2 in 100 gastrointestinal cancerous tissues. IFNAR2 expression was examined using immunohistochemistry, in surgically resected tissue samples (20 esophageal, 20 gastric, 20 colorectal, 20 cholangiocarcinoma, and 20 pancreatic samples). The expression rate of IFNAR2 was 35.0% (7/20), 25.0% (5/20), 20.0% (4/20), 45.0% (9/20), and 25.0% (5/20) in esophageal, gastric cancer, colorectal, cholangiocarcinoma and pancreatic cancer samples, respectively. In our previous report, the expression rate of IFNAR2 in HCC samples was 64.8% (59/91). Thus, the expression rates of IFNAR2 in the five types of gastrointestinal cancers tested here were low, compared with HCC. The clinical efficacy of IFN-α mono- or combination therapies in patients with gastrointestinal neoplasms is expected to be lower than in patients with HCC based on the expression level of IFNAR2.

Correspondence to: Dr Hiroaki Nagano, Department of Surgery, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
E-mail: hnagano@surg2.med.osaka-u.ac.jp

Abbreviations: IFN, interferon; IFNAR2, type I interferon-α/ß receptor 2; HCC, hepatocellular carcinoma; 5-FU, 5-fluorouracil

Key words: gastrointestinal cancer, IFNAR2, IFN-α, therapy, prediction

Introduction

Interferon (IFN)-α is used in the treatment of several neoplasms and viral disorders such as chronic myeloid leukemia, hairy-cell leukemia, adult T cell leukemia, multiple myeloma, renal cell carcinoma, and chronic hepatitis B and C. For example, Kantarjian *et al* (1) reported that major cytogenetic responses occurred in 37% of 148 treated chronic myeloid leukemia patients. Furthermore, the myeloma trialists collaborative groups (2) demonstrated complete and partial responses to IFN monotherapy in 57.5% of patients with myeloma in meta-analysis study. IFN-α therapy for these diseases is associated with a good prognosis. Based on these findings, IFN-α therapy has been introduced in patients with colorectal cancer. However, Figlin *et al* (3) reported no responses in 18 patients with colorectal adenocarcinoma, as did Silgals *et al* (4) in 15 patients with colorectal cancer. However, Eggermont *et al* (5) reported a 10% response rate in 10 patients with colorectal cancer. From these reports, it is plausible that IFN-α monotherapy might be effective in patients with colorectal carcinoma. In addition, IFN-α has been used in combination with several anti-cancer agents to enhance the treatment effect. However, this treatment effect was not expected in gastrointestinal cancers and remains controversial, even in colorectal cancer.

IFN-α therapy has also been introduced in hepatocellular carcinoma (HCC). In several randomized controlled trials, IFN-α significantly improved survival in patients with unresectable HCC (6, 7). Moreover, combination therapy with IFN-α has been used for HCC with good efficiency (8-14). In a randomized control trial from Hong Kong, IFN-α treatment was associated with a significant increase in major and minor responses (22%) in comparison to doxorubicin (0%) (15). However, occasional dramatic responses were not seen in 5-fluorouracil (5-FU) monotherapy of patients with HCC (16-18).

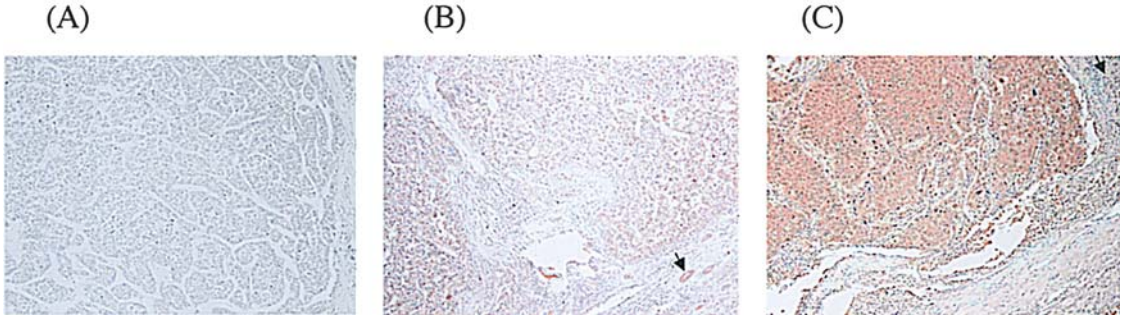

Figure 1. Immunohistochemistry of IFNAR2 expression in HCC tissues as control samples. Representative tissues were used as control samples. The intensity of IFNAR2 was scored on a scale from 0 to 2; 0, no or faint staining (A); 1, moderate staining (B); and 2, strong staining (C). The latter level of staining was used as an inner control within the sample, which was designated arbitrarily as intensity 1, because the epithelial cells of the bile ducts generally expressed moderate levels of IFNAR2.

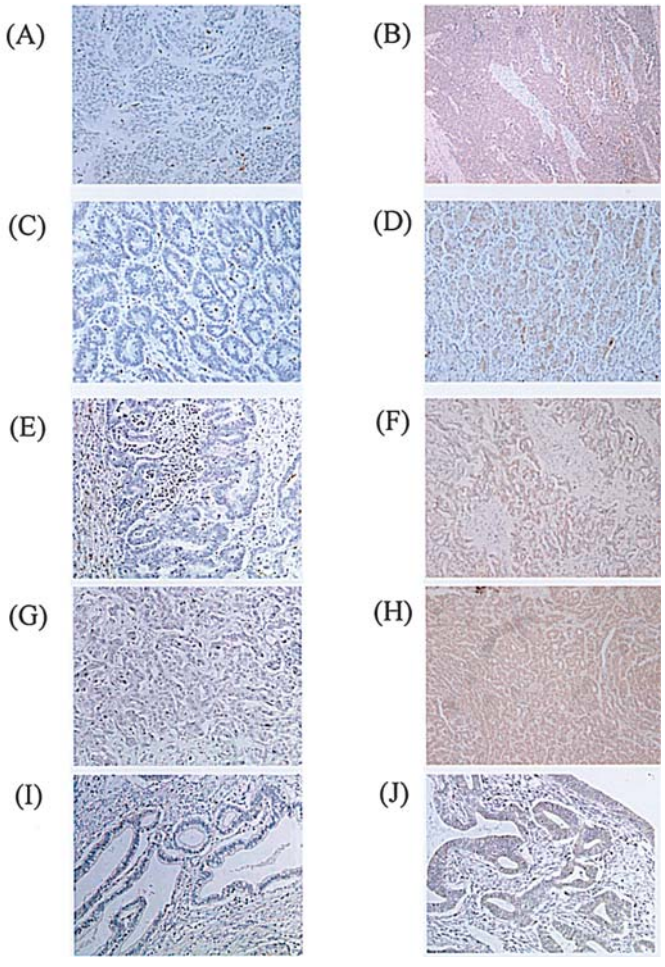

Figure 2. Immunohistochemical analysis of IFNAR2 expression in cancerous tissue. The intensity of IFNAR2 was scored from 0 to 2 simultaneously with representative tissues of staining intensity of 0 to 2 in HCC. (A, B) Esophageal cancers; intensity 0 (A) and intensity 2 (B). (C, D) Gastric cancers; intensity 0 (C) and intensity 1 (D). (E, F) Colorectal cancers; intensity 0 (E) and intensity 2 (F). (G, H) Cholangiocellular carcinomas; intensity 0, (G) and intensity 2 (H). (I, J) Pancreatic cancers; intensity 0 (I) and intensity 2 (J).

Based on these reports, the clinical use of IFN-α in combination with cytotoxic drugs, such as 5-FU, cisplatin, methotrexate, doxorubicin, may result in beneficial effects in HCC patients.

The mechanism of IFN-α therapy involves binding of IFN-α to type I IFN-receptors (IFNAR1 and IFNAR2 long forms), and binding to the promoter elements of type I IFN-induced genes to initiate their transcription (19-21), resulting in apoptosis. In these signal transduction pathways, IFN receptors play an important role in the treatment of several neoplastic disorders. It thus follows that IFN-α resistance is associated with a lack of IFN receptors. We recently reported that IFNAR2 expression significantly correlates with the effects of IFN-α/5-FU combination therapy in HCC (22). Therefore, the expression of IFNAR2 may be a useful predictive factor for the effect of IFN-α therapy.

In the present study, we investigated the expression of IFNAR2 as a predictive marker for the therapeutic effect of IFN-α on the progression of gastrointestinal cancers.

Materials and methods

Tumor samples. One hundred surgically resected gastrointestinal cancer samples [20 esophageal, 20 gastric cancer, 20 colorectal (including 9 patients with colorectal liver metastasis), 20 cholangiocarcinoma, and 20 pancreatic] were used in this study. All patients underwent surgery at the Department of Surgery, Osaka University Hospital, between December 1997 and December 2004. Expression of the IFN-receptor was examined by immunohistochemistry. The study protocol was approved by the Human Ethics Review Committee of Osaka University.

Immunohistochemistry. Immunohistochemistry was carried out using the method described in our previous report (23). Tissue sections (4-μm thick) were deparaffinized in xylene and heat antigen retrieval was performed as described previously (23). The slides were then processed for immunohistochemistry on the TechMate Horizon automated staining system (Dako, Glostrup, Denmark) (24), using the EnVision+ peroxidase kit (Dako) (25). In the step of primary antibody reaction, the slides were incubated with the IFNAR2 antibody (final concentration: 2.5 mg/ml) (Otsuka Pharmaceutical Co., Tokushima, Japan) overnight at 4˚C. For negative controls, non-immunized rabbit IgG (Vector Laboratories, Burlingame, CA) or Tris buffered saline (TBS) was used as a substitute for the primary antibody to verify the possibility of false positive

xpression of IFNAR2 in gastrointestinal cancers.

	Number of cases	Intensity			Expression rate	Strong expression rate
		0	1	2		
Esophageal cancer	20	13	6	1	35.0%	5.0%
Gastric cancer	20	15	5	0	25.0%	0%
Colorectal cancer	20	16	3	1	20.0%	5.0%
Cholangiolar carcinoma	20	11	8	1	45.0%	5.0%
Pancreatic cancer	20	15	3	2	25.0%	10.0%
Hepatocellular carcinoma[a]	91	32	35	24	64.8%	26.4%

[a]As previously reported (23).

Table II. Randomized control trials of 5-FU and interferon-α in colorectal cancer.

Author	Regimen	Dose of 5-fluorouracil	Dose of IFN-α	Response rate
Hill *et al* (33)	5-FU/LV/IFN-α	750 mg/m^2 i.v. Day 1-5	10 MU/m^2 i.m. 3 times/week	19% (10/52)
	5-FU alone	750 mg/m^2 i.v. Day 1-5	-	30% (16/54)
Kosmidis *et al* (34)	5-FU/LV/IFN-α	450 mg/m^2 i.v. Day 1-5	5 MU/m^2 i.m. 3 times/week	9.8% (5/53)
	5-FU/LV	450 mg/m^2 i.v. Day 1-5	-	7.8% (4/53)
Palmeri *et al* (35)	5-FU/IFN-α	750 mg/m^2 i.v. Day 1-5	3 MU/m^2 i.m. 3 times/week	25% (25/101)
	5-FU alone	750 mg/m^2 i.v. Day 1-5	-	21% (22/104)
Hausminger *et al* (36)	5-FU/LV/IFN-α	500 mg/m^2 i.v. Day 1-5	7 MU/m^2 i.m. 3 times/week	36% (38/107)
	5-FU/LV	500 mg/m^2 i.v. Day 1-5	-	25% (28/112)
Colucci *et al* (37)	5-FU/LV/IFN-α	350 mg/m^2 i.v. Day 1-5	3 MU/m^2 sc. 5 consecutive days/week	24% (25/103)
	5-FU/LV	350 mg/m^2 i.v. Day 1-5	-	23% (23/101)

5-FU, 5-fluorouracil; LV, leucovorin; IFN, interferon.

responses from non-specific binding of IgG or from the secondary antibody. In addition, absorption tests were performed on tissue sections. The intensity of IFNAR2 was scored in a scale from 0 to 2, arbitrarily in 91 HCC samples as reported previously (23). HCC samples including one of no staining (Fig. 1A), one of moderate staining (Fig. 1B) and one of strong staining (Fig. 1C) were used as control samples. All 100 samples with gastrointestinal cancer, except HCC, were evaluated by comparing with these three representative control samples. IFNAR2 expression was often heterogeneous. The histological or immunohistological type that constituted the major volume of the tumor was selected as the representative type. Staining was repeated at least twice to avoid possible technical errors and identical results were obtained. All slides were interpreted by an investigator blinded to the clinical and pathological parameters.

Results

All 100 samples with gastrointestinal cancers, except HCC, were evaluated by comparison with tissue samples of no staining (Fig. 1A), moderate staining (Fig. 1B), and strong staining in HCC (Fig. 1C) as control samples. In the 20 samples of esophageal cancer, strong expression of IFNAR2 was observed in 5.0% (1/20) of the samples; 1 strongly, 6 moderately, and 13 without or faintly stained (Fig. 2A and B). In the 20 samples of gastric cancer, strong expression of IFNAR2 was observed in 0% (0/20) of the samples; 5 moderately and 15 without or faintly stained (Fig. 2C and D). In the 20 samples of colorectal cancer (including 9 colorectal liver metastasis samples), strong expression of IFNAR2 was observed in 5.0% (1/20) of samples; 1 strongly, 3 moderately, and 16 without or faintly stained (Fig. 2E and F). In the 20 samples of cholangiocellular carcinoma, strong expression of IFNAR2 was observed in 5.0% (1/20) of samples; 1 strongly, 8 moderately, and 11 without or faintly stained (Fig. 2G and H). In the 20 samples of pancreatic cancer, strong expression of IFNAR2 was observed in 10.0% (2/20) of samples; 2 strongly, 3 moderately, and 15 without or faintly stained (Fig. 2I and J) (Table I). In comparison, we have previously reported that the expression of IFNAR2 in HCC was observed in 26.4% (24/91) of the samples; 24 strongly, 35 moderately, and 32 without or faintly stained (23).

Table III. Interferon-α therapy in gastrointestinal cancers.

Cancer	Author/(Refs.)	Dose of IFN-α	Dose of 5-fluorouracil	Doses of other drugs	One term (weeks)	Response %
Colorectal cancer	Figlin *et al* (3)	3 MU/day i.m. 5 days/week	-	-	2	0 (0/18)
	Silgals *et al* (4)	30-50 MU/m^2 i.v. 5 days/week	-	-	2-3	0 (0/15)
	Eggermont *et al* (5)	20 MU/m^2 i.m. twice a week	-	-	12	10 (1/10)
	Wadler *et al* (26)	9 MU/m^2 sc. three times/week	750 mg/m^2 i.v. Day 1-5, continuous	-	-	76 (13/17)
	Wadler *et al* (27)	9 MU/m^2 i.m. three times/week	750 mg/m^2 i.v. Day 1-5, continuous	-	4	63 (23/32)
	Wadler *et al* (28)	15-18 MU/m^2 i.m. Day 1,3,5	750 mg/m^2 i.v. Day 1-5, continuous	-	-	42 (15/36)
	Hill *et al* (33)	10 MU/m^2 i.m. 3 times/week	750 mg/m^2 i.v. Day 1-5	-	2	19 (10/52)
	Kosmidis *et al* (34)	5 MU/m^2 i.m. 3 times/week	450 mg/m^2 i.v. Day 1-2/week	Leucovorin (200 mg/m^2 i.v)	8	9.8 (5/53)
	Palmeri *et al* (35)	3 MU/m^2 i.m. Day 1,3,5/week	750 mg/m^2 i.v. Day 1-5	-	4	25 (25/101)
	Hausminger *et al* (36)	7 MU/m^2 sc. 3 times/week	500 mg/m^2 i.v. Day 1-5	Leucovorin (100 mg/m^2 i.v)	10	36 (38/107)
	Colucci *et al* (37)	3 MU/m^2 sc. 5 consecutive days/week	350 mg/m^2 i.v. Day 1-5	Leucovorin (200 mg/m^2 i.v)	3	24 (25/103)
Esophageal cancer	Kelsen *et al* (39)	9 MU/m^2 sc. 3 times/week	750 mg/m^2 i.v. Day 1-5 continuous, Day 12 bolus	-	at least 1 month	27 (10/37)
	Wadler *et al* (40)	9 MU/m^2 sc. 3 times/week	750 mg/m^2 i.v, Day 1-5/week	-	6	25 (5/20)
	Ilson *et al* (41)	3 MU/m^2 sc. Day 1-2/week	750 mg/m^2 i.v. Day 1-5	CDDP (100 mg/m^2 i.v. Day 1)	4	50 (13/26)
	Wadler *et al* (42)	10 MU/m^2 sc. 3 times/week	750 mg/m^2 i.v, Day 1-5/week	CDDP (100 mg/m^2 i.v. Day 1)	4	65 (15/23)
Gastric cancer	Lee *et al* (43)	5 MU/m^2 i.m. Day 1-7/week	750 mg/m^2 i.v. Day 2-6	-	4	30.7 (4/13)
	Hudes *et al* (44)	5 MU/m^2 i.m. Day 1-7/week	370 mg/m^2 i.v. Day 2-6	Leucovorin (500 mg/m^2 i.v. Day 2-6)	4	12.5 (3/24)
	Wadler *et al* (45)	9 MU/m^2 i.m. 3 times/week	2600 mg/m^2 i.v, continuous. Day 1,8,15,22,29,36	Hydroxyurea (4300 mg/m^2 iv. Day 1,8,15,22,29,36)	6	37 (11/31)
Pancreatic cancer	Bernard *et al* (47)	6 MU/m^2 i.m. Day 1,8,15,22	500 mg/m2 i.v. Day 1,8,15,22	Folinic acid (500 mg/m^2 i.v. Day 1,8,15,22)	4	14.0 (8/57)
	Wadler *et al* (45)	9 MU/m^2 i.m. 3 times/week	2600 mg/m2 i.v, continuous. Day 1,8,15,22,29,36	Hydroxyurea (4300 mg/m^2 iv. Day 1,8,15,22,29,36)	6	4.7 (1/21)
	Wagener *et al* (46)	3 MU/m^2 s.c. Day 1-5/week	1000 mg/m^2 i.v. continuous, Day 1-5/week	CDDP (100 mg/m^2 i.v. Day 1/week)	4	13.3 (2/15)
Hepatocellular carcinoma	Lai *et al* (6)	5 MU/m^2 i.m. 3 times/week	-	-	1	31 (11/35)
	Yuen *et al* (7)	10 or 30 or 50 MU/m^2 TAI	-	-	8-12	64.3 (9/14)
	Patt *et al* (14)	5 MU/m^2 sc. 3 times/week	750 mg/m^2 i.a. Day 1-5/week	-	4	21 (6/28)
	Urabe *et al* (8)	3 MU/m^2 sc. Day 1,3,5/week	750 mg/m^2 i.a. weekly	CDDP (75 mg/m^2 i.a. , every 2 weeks) MTX (30 mg/m^2 i.a. every 4 weeks) leucovorin (30 mg/m^2 i.v. every 4 weeks)	4	47 (7/15)
	Leung *et al* (10)	5 MU/m^2 sc. Day 1-4/week	400 mg/m^2 i.v. Day 1-4	CDDP (20 mg/m^2 i.v. Day 1-4, every week), Doxorubicin (40 mg/m^2 i.v. Day 1)	3	26 (13/50)
	Chung *et al* (11)	5 MU/m^2 i.m. 3 times/week	-	CDDP (2 mg/kg continuous i.a. every 8 weeks)	8	33 (6/18)
	Sakon *et al* (13)	5 MU/m^2 sc. 3 times/week	300 mg/m^2 i.a. Day 1-5/week	-	4	63 (8/13)
	Patt *et al* (9)	4 MU/m^2 sc. 3 times/week	200 mg/m^2 i.v. Day 1-21	-	4	25 (9/36)

IFN, interferon; 5-FU, 5-fluorouracil; CDDP, cisplatin; MTX, methotrexate.

Discussion

IFN-α had been proposed to increase the efficacy of 5-FU in many single arm trials of IFN-α/5-FU combination therapy for colorectal carcinoma. For example, Wadler *et al* (26) reported that response rates were 76% (13/17) in untreated patients with advanced colorectal cancer. In 1990, they indicated that response rates were 63% in 32 untreated patients with advanced colorectal cancer (27). This combination therapy produced objective responses in 15 of 36 (42%) patients with colorectal carcinoma in an Eastern Cooperative Oncology Group (ECOG) study (28). In contrast, 5-FU monotherapy induced objective remissions in only 3 to 25% of patients with few complete responders, few durable remissions, and no improvement in overall survival (29-32). These results suggest that IFN-α/5-FU combination therapy may generate beneficial effects in colorectal cancer patients (Table II).

However, it was not clear whether IFN-α increases the efficiency of 5-FU in the treatment of colorectal cancer. Therefore, many randomized controlled trials comparing the combination of 5-FU and IFN-α to 5-FU alone have been performed. Hill *et al* (33) indicated that IFN adds no benefit to

terms of response rates and survival and significantly toxicity in patients with advanced colorectal cancer. Kosmidis *et al* (34) reported that the addition of IFN-α2b to the combination of 5-FU and folinic acid contributes to decreased survival. Palmeri *et al* (35) noted significantly longer survival in patients who achieved a complete response after IFN-α therapy; however, overall survival was not affected. Hausminger *et al* (36) reported that the addition of IFN to 5-FU/Leucovorin (LV), in schedules and doses used in the present study, did not provide any clinical benefit over 5-FU/LV. Colucci *et al* (37) reported that no differences in the objective response rate, median duration of response, time to progression, and median survival [comparison between combination of levofolinic acid (l-FA)/5-FU and combination of l-FA/5-FU/IFN]. In a meta-analysis of these randomized control trials, IFN-α was not found to increase the efficiency of 5-FU in advanced colon cancer (38). Moreover, many therapies combined with IFN-α have been introduced for other gastrointestinal cancers. However, in common with colorectal cancer, the clinical use of IFN-α for other gastrointestinal cancers is doubtful (39-47) (Table III).

Several mechanisms for the anti-cancer effects of IFN-α have been proposed and can be direct and/or indirect anti-tumor effects. The direct anti-tumor effects include cell damage (48), upregulation of cancer antigens (49), and delayed action on the cell cycle (50). In contrast, indirect anti-tumor actions include activation of natural killer cells (51), T cells (52), and macrophages (53). IFN-α induces cyclin-dependent kinase inhibitors involved in G1/G0 arrest (54). IFN-α may also exert its anti-tumor effect indirectly via the immune system since it is known to augment T-cell cytotoxicity (55,56). Recently, we demonstrated that the modulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor-mediated cytotoxic pathway might contribute to the anti-HCC effect of IFN-α/5-FU combination therapy (57). Furthermore, IFN-α induces apoptosis of various cancer cells (58). Another possible mechanism of action is via its anti-angiogenesis activity (59).

Based on these anti-tumor mechanisms of IFN-α, IFN-α suppressed the proliferation of all IFNAR2-positive cancer cell lines *in vitro* through mechanisms related to apoptosis or inhibition of the cell cycle (60). The importance of IFNAR2 expression for the anti-cancer effect of IFN-α injection was also reported in our recent studies (61,62). These findings suggest that the anti-neoplastic effects of IFN-α are mediated through IFNAR2. Recently, we found that the expression of IFNAR2 significantly correlates with IFN-α/5-FU combination therapy effects (22) and IFNAR2-induced signal transduction was useful for molecular prediction of the response to IFN-α/ 5-FU combination therapy in advanced HCC (63). Therefore, this significant relationship demonstrated in HCC should be also present in other gastrointestinal cancers. Accordingly, we investigated IFNAR2 expression in various gastrointestinal cancers using immunohistochemistry, as described in our previous report (23). The expression rate of IFNAR2 was not high in gastrointestinal cancers, in comparison with our previous results in HCC. We previously reported a strong expression rate of IFNAR2, at 26.4% (35/91), in HCC (23). The results of the present study indicate that the strong expression rate of IFNAR2 in gastrointestinal cancers, excluding HCC, was within 10% and lower than that in HCC. Based on these results, we believe that the efficiency of combination therapy with IFN-α in these cancers is expected be lower than that in HCC.

These results were compatible with those reported by Ambrus *et al* (64). They reported that all patients had increased levels of free interferon receptor-α/ß type-I in the circulation, with the highest levels reported in patients with adenocarcinoma. High IFN inhibitory activity in patients with cancer may be a significant factor in their increased susceptibility to progressive disease. These soluble forms can be both agonists and antagonists, depending on their concentration (65), and high levels of circulating soluble IFN receptors may block the anti-proliferative activity of IFN-α in adenocarcinomas.

In summary, we demonstrated that IFNAR2 expression rates in esophageal, gastric, colorectal, and pancreatic cancers and cholangiocarcinoma were lower than in HCC. Compared with our recent investigation in HCC, the clinical efficacy of IFN-α in combination therapies with other cytotoxic drugs in these gastrointestinal cancers is expected to be lower than that in HCC. To increase the treatment efficacy of IFN for gastrointestinal cancers other than HCC, other treatment modalities should be included, such as IFNAR2 gene transfection (66) or IFN-based chemoradiation (67).

Acknowledgements

The authors thank Dr Yasukazu Ohmoto from the First Institute of New Drug Research, and Otsuka Pharmaceutical Co., Ltd., for providing anti-human IFNAR2 antibody (OCT4813). The authors gratefully acknowledge Mrs. Satomi Yamane for her excellent technical assistance. This work was supported by a Grant-in-Aid for cancer research from the Ministry of Education, Culture and Science of Japan.

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