—Original—

Expression of Fibroblast Growth Factor (FGF)-10 in Human Colorectal Adenocarcinoma Cells

Aihiko Matsuike[1], Toshiyuki Ishiwata[1], Masanori Watanabe[2] and Goro Asano[1]

[1]Department of Pathology, Nippon Medical School
[2]Center for Digestive Diseases, Second Hospital, Nippon Medical School

Abstract

Fibroblast growth factor (FGF)-10 (keratinocyte growth factor 2, KGF 2) is a new member of the FGF family that is mainly synthesized by mesenchymal cells and acts predominantly on epithelial cells in a paracrine manner. Its actions are dependent on its binding to the iiib isoform of the cell-surface FGF receptor 2 (FGFR 2 iiib). FGF-10 is known to play an important role in fetal limb and lung development, skin wound healing and prostatic epithelial cell growth.

In the present study, the expression of FGF-10 and FGFR 2 iiib in five cultured human colorectal adenocarcinoma cell lines (COLO 205, DLD-1, HCT-15, SW 480 and WiDr) and the localization of FGF-10 messenger RNA (mRNA) and its protein in human colorectal cancer tissues from 10 patients were determined. All five colorectal cancer cell lines expressed FGF-10 mRNA and its protein. FGFR 2 iiib mRNAs were expressed in these cells and the recombinant FGF-10 (1 ng/ml) increased the growth rate of COLO 205 cells. To determine the localization of FGF-10 protein and its mRNA in normal and cancerous human colorectal tissues, immunohistochemistry and in situ hybridization were performed. In normal colorectal tissues, FGF-10 and its mRNA were not detected. In contrast, moderate immunoreactivity was present in cancer cells in 5 of 10 colorectal cancer cases and mild immunoreactivity was recognized in adjacent fibroblasts. By using in situ hybridization, FGF-10 mRNA was observed in colorectal cancer cells and fibroblasts adjacent to cancer cells.

These findings indicate that FGF-10 and its receptor, FGFR 2 iiib expression in colorectal adenocarcinoma cells and FGF-10 may contribute to the growth of cells of this type.
(J Nippon Med Sch 2001; 68: 397—404)

Key words: FGF-10, colorectal cancer, RT-PCR, in situ hybridization, immunohistochemistry

Introduction

The incidence of colorectal adenocarcinoma has been increasing in Japan as well as in the Western world[1]. A high percentage of cells from patients with this cancer overexpress a number of growth factors and their receptors, including the transforming growth factor-beta (TGF-beta), insulin-like growth factor-II (IGF-II), hepatocyte growth factor (HGF), HGF receptor (c-Met), epidermal growth factor receptor (EGFR), IGF receptor I (IGF-IR) and vascular endothelial growth factor (VEGF)[2-8].

Fibroblast growth factor (FGF)-10 is a new member

Correspondence to Toshiyuki Ishiwata, MD, Department of Pathology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
E-mail: ishiwata@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

of the FGF family of heparin-binding polypeptides that was originally isolated from the rat embryo cDNA as determined by homology-based polymerase chain reaction[9,10]. FGF-10 is mainly synthesized by mesenchymal cells and acts predominantly on epithelial cells in a paracrine manner[11]. Moreover, FGF-10 actions are dependent on its binding to the iiib isoform of the cell-surface FGF receptor 2 (FGFR 2)[12]. FGF-10 is known to be expressed in the normal lung, heart, skin, brain, and prostate[9-13]. In addition to FGF-10, which is also known as keratinocyte growth factor (KGF)-2, this family includes aFGF or FGF-1, bFGF or FGF-2, int-2 (FGF-3), hst/K-FGF (FGF-4), FGF-5, FGF-6, FGF-7, androgen-induced growth factor (AIGF or FGF-8), glia-activating factor (GAF or FGF-9), FGF-11 (FGF homologous factors-3, FHF-3), FGF-12 (FHF-1), FGF-13 (FHF-2), FGF-14 (FHF-4), FGF-15, FGF-16, FGF-17, FGF-18 and FGF-19[14-20]. In the FGF family, we previously reported that basic FGF (bFGF, FGF-2) and FGF-7 (also known as keratinocyte growth factor, KGF) were overexpressed in cells from patients with colorectal cancer[2,21]. Recently we found that FGF-10 was expressed in a human squamous carcinoma cell line from the uterine cervix[22]. However, it has not been clarified whether adenocarcinoma cells express FGF-10. Therefore, in the present study, we examined the FGF-10 mRNA expression in cultured human colorectal adenocarcinoma cell lines and in colorectal cancer tissues. We now report that FGF-10 mRNA, its protein and FGFR 2 iiib mRNA are expressed in five cultured colorectal adenocarcinoma cell lines and that the recombinant FGF-10 stimulates growth of COLO 205 colorectal cancer cells. In human colorectal cancer tissues, FGF-10 is expressed in some cancer cells and is also localized in fibroblasts adjacent to cancer cells.

Materials and Methods

Materials:

The following were purchased: pGEM-T Easy vector from Promega Biotech. (Madison WI, USA); a Takara RNA polymerase chain reaction (PCR) kit (AMV) Ver. 2.1 from Takara (Tokyo, Japan); Isogen from Nippon Gene (Tokyo, Japan); Pefabloc SC from Merck (Darmstadt, Germany); anti-rabbit IgG-HRP secondary antibody, the rabbit anti-FGF-10 polyclonal antibody (H-121) and a blocking peptide from Santa Cruz Biotech. (Santa Cruz, CA, USA); a universal VECTASTAIN ABC kit from Vector Lab. Inc. (Burlingame, CA, USA); human recombinant FGF-10 from R&D System Inc. (Westerville, OH, USA); yeast t-RNA from GIBCO BRL (Gaithersbung, MD); WST-1, Tween 20, glycine and formamide from Wako Pure Chemical Industries Ltd. (Osaka, Japan); DIG nucleic acid detection kit and DIG RNA labeling kit from Roche Diagnostics (Mannheim, Germany); cell culture plates from Nalge Nunc Int. (Roskilde, Denmark); IPVH membranes from Millipore (Yonezawa, Japan); SuperSignal West Pico Chemiluminescent substrate from Pierce Chemical Company (Rockford, IL); MAS-coated slides from Matunami Glass Ind. Ltd. (Osaka, Japan); all other chemicals and reagents from Sigma Chemical Corp. (St. Louis, MO, USA).

Tissue samples:

Colorectal carcinoma tissue samples were obtained from patients (4 males, 6 females; mean age, 70 years; range 58 to 85 years) undergoing proctocolectomy for colorectal cancer at the Center for Digestive Diseases, Second Affiliated Hospital of Nippon Medical School. Normal colorectal tissue samples were also obtained from the surgical margin at least 10 cm away from the cancerous portion in the same patients. The tissues were fixed in 10% paraformaldehyde solution (PFA) for 18～20 hr and embedded in paraffin.

Cell culture and growth assay:

COLO 205, DLD-1, HCT-15, SW 480 and WiDr cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The cells were grown in the RPMI 1640 medium containing 10% heat-inactivated bovine serum (FBS), 0.2% glucose, 1 mM pyruvate, 2 mM glutamine, 100 unit/ml penicillin, 100 mg/ml streptomycin and 60 mg/ml kanamycin at 37℃ under a humidified 5% CO_2 atmosphere. To assess the effects of FGF-10 on colon cancer cell growth, COLO 205 cells were plated at a density of 10,000 cells per well in a 96-well plate and grown overnight in the RPMI 1640 medium supplemented with 10% FBS. Then, the cells were incubated in the absence or presence of human recombinant FGF-10 and harvested for 24 hr in serum-free RPMI 1640 me-

dium. The cells were further incubated in the same medium with WST-1 for 2 hr and the optical density of the culture solution in the plate was measured using an ELISA plate reader (Bio Rad Lab. Hercules, CA) at 415 nm. Data were expressed as percent increase with respect to those of untreated controls and were the mean ± s.d of three separate experiments.

RT-PCR (Reverse transcription-polymerase chain reaction):

Total RNAs were extracted from colorectal cancer cells with Isogen according to the manufacturer's protocol. Then cDNA synthesis and PCR were performed with a Takara RNA PCR kit. The primer pair used for FGF-10 amplification corresponded to nucleotides (nts) 77 − 96 (5'-CGC-GGA-TCC-TGC-TGT-TCT-TGG-TGT-CTT-CC-3') and nt 276 − 296 (5'-CGG-AAT-TCT-GAC-CTT-CCC-GTT-CTT-CTC-A-3') of the human FGF-10 cDNA (238 bp)[10]. The primer pair used for FGFR 2 iiib mRNA amplification corresponded to nt 1346 − 1365 (5'-CGC-GGA-TCC-GCC-GCC-GGT-GTT-AAC-ACC-AC-3') and nt 1456 − 1475 (5'-CGG-AAT-TCA-CCA-TGC-AGA-GTG-AAA-GGA-T-3'; 149 bp)[21].

PCR reaction was carried out in a Takara PCR thermal cycler MP (Takara, Tokyo, Japan) for 5 min at 95℃ and then 35 cycles, each consisting of 1 min at 95℃, 1 min at 58℃ and 2 min at 72℃. As a negative control, RNA without reverse transcription was used as the template of PCR.

Western blot analysis:

The anti-FGF-10 antibody used for Western blot analysis was an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to amino acids 10 − 130 mapping with an internal region of human FGF-10. This antibody reacts with human FGF-10 on Western blotting, immunoprecipitation and immunohistochemistry, but does not react with any other members of the FGF family (Santa Cruz Biotech., CA). Protein extraction was performed as previously reported[23]. The proteins extracted were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to IPVH membranes, which were then incubated for 16 hr with the FGF-10 antibody. The membranes were sequentially washed and incubated with secondary HRP conjugated anti-rabbit IgG antibody for 2 hr. After washing, antibodies were visualized by enhanced chemiluminescence.

Immunohistochemistry:

The same anti-FGF-10 antibody used for Western blot analysis was employed for immunohistochemistry. Paraffin-embedded sections (3 μm) were subjected to immunostaining by the avidin-peroxidase technique. After deparaffinization, the tissue sections were incubated in 0.1% trypsin solution for 60 min at 37℃. Endogenous peroxidase activity was blocked by incubation for 30 min with 0.3% hydrogen peroxide in methanol. Tissue sections were incubated with normal horse serum for 10 min at room temperature (RT), then with FGF-10 (1: 500 in dilution) in PBS containing 1% bovine serum albumin (BSA) for 16 hr at 4℃. Bound antibodies were detected with biotinylated horse universal (anti-rabbit and mouse) IgG secondary antibodies and the streptavidin-peroxidase complex, using diaminobenzidine tetrahydrochloride as the substrate. Sections were counterstained with Mayer's hematoxylin.

Probe preparation:

A BamHI-EcoRI cDNA fragment, corresponding to nts 77 − 295 of the human FGF-10 cDNA sequence, was generated by PCR amplification of a single-stranded cDNA that was reverse-transcribed (RT) from human placental RNA, as previously described[24]. The FGF-10 cDNA fragments were subcloned into the pGEM-T Easy vector. The authenticity of these fragments was confirmed by sequencing. The probes were labeled with digoxigenin-UTP using SP 6 or T 7 RNA polymerase using a DIG RNA labeling kit.

In situ hybridization:

In situ hybridization was performed as previously reported[25]. Tissue sections were placed on MAS-coated slides, deparaffinized and incubated at RT for 20 min with 0.2 N HCl and at 37℃ for 15 min with 100 μg/ml proteinase K. The sections were then postfixed for 5 min in phosphate-buffered saline (PBS) containing 4% PFA, incubated briefly twice in PBS containing 2 mg/ml glycine and once in 50% (vol/vol) formamide/2 X SSC for 1 hr prior to the initiation of hybridization reaction by the addition of 100 μl of hybridization buffer. In the case of FGF-10, hybridization was performed in a moist chamber for 16 hr at 42℃ at a probe concentration of 100 ng/ml. The sections were

then washed sequentially with 2 X SSC for 20 min at 42℃ and 0.2 X SSC for 20 min at 42℃. For immunological detection, a DIG nucleic acid detection kit was used. The sections were washed briefly with buffer 1 solution (100 mM Tris-HCl and 150 mM NaCl, pH 7.5) and incubated with 1% (wt/vol) blocking reagent in the same buffer 1 solution for 60 min at RT. They were then incubated for 30 min at RT with a 1: 2000 dilution of alkaline phosphatase-conjugated polyclonal sheep anti-digoxigenin Fab fragment containing 0.2% Tween 20. The sections were then washed twice for 15 min at 23℃ with buffer 1 solution containing 0.2% Tween 20 and incubated in buffer 3 solution (100 mM Tris-HCl, 100 mM NaCl and 50 mM $MgCl_2$, pH 9.5) for 2 min. The sections were then incubated with a color solution containing nitroblue tetrazolium and X-phosphate in a dark box for 1－2 hr. The reaction was stopped with a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and the sections were mounted in an aqueous mounting medium.

Results

RT-PCR:

To examine the expression of FGF-10 and its receptor, FGFR 2 iiib (KGFR) mRNA in cultured colorectal cancer cell lines, RT-PCR analysis was performed. All five cultured colorectal adenocarcinoma cell lines, COLO 205, DLD-1, HCT-15, SW 480 and WiDr cells expressed FGF-10 mRNA (**Fig. 1,** upper panel) and FGFR 2 iiib mRNA (lower panel).

Western blot analysis:

To confirm the synthesis of FGF-10 protein in five cultured colorectal cancer cell lines, Western blot analysis was performed. Approximately 30 kD band corresponding to FGF-10 protein was detected in all five cultured colorectal cancer cells (**Fig. 2**).

Effect of FGF-10 on COLO 205 colorectal cancer cells:

We next determined whether FGF-10 modulated the growth of COLO 205 cells. In comparison to the untreated control, maximal stimulation occurred at 1 ng/ml recombinant FGF-10 enhancing the growth of COLO 205 cells by 26% (**Fig. 3**).

Immunohistochemistry:

Immunohistochemical staining was performed in order to determine the localization of FGF-10 in colorectal cancer tissues. FGF-10 was not observed in normal colon tissues (**Fig. 4A**), but was moderately observed in cancer cells in five of ten cancer cases (**Fig. 4B**, arrows). And mild to moderate FGF-10 immunoreactivity was present in fibroblasts adjacent to cancer cells (**Fig. 4B, C,** arrows).

In situ hybridization:

To determine which the cell types express FGF-10 mRNA in colorectal cancer tissues, in situ hybridization was employed. FGF-10 mRNA was not detected in normal colorectal tissues (data not shown), while it was strongly expressed in most of the cytoplasm of cancer cells in immunohistochemically FGF-10-positive cancer cases (**Fig. 5A** arrows, **B**). In situ hybridization with a sense FGF-10 probe did not produce any specific signal (**Fig. 5C**).

Discussion

FGF-10 is generally synthesized by stromal cells

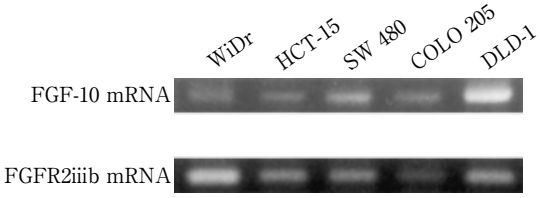

Fig. 1 RT-PCR analysis of colorectal adenocarcinoma cell lines.
All five cancer cell lines, WiDr, HCT-15, SW 480, COLO 205 and DLD-1 express FGF-10 mRNA (upper panel) and FGFR 2 iiib mRNA (lower panel).

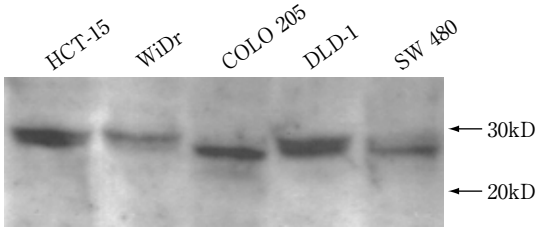

Fig. 2 Western blot analysis of FGF-10 in colorectal adenocarcinoma cell lines.
Approximately 30 kD band corresponding to FGF-10 protein is detected in HCT-15, WiDr, COLO 205, DLD-1 and SW 480, colorectal cancer cells.

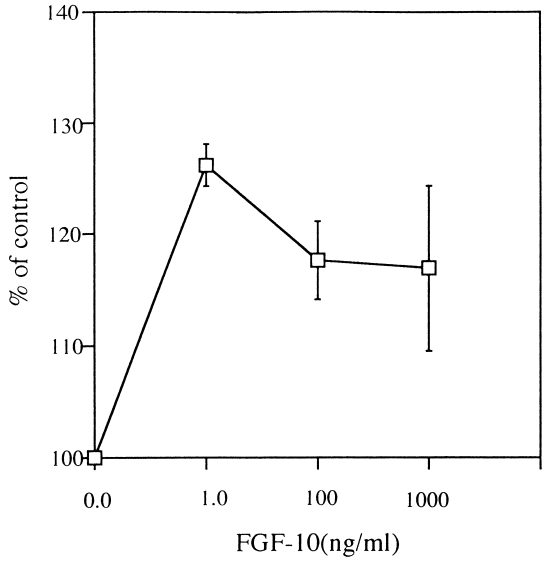

Fig. 3 Effects of FGF-10 on COLO 205 colorectal cancer cell growth.
COLO 205 cells were grown in a serum-free RPMI medium in the presence or absence of increasing concentrations of FGF-10. Data are expressed as percent increase with respect to those of untreated controls and are the means ± s.d of three separate experiments.

and stimulates epithelial cell growth. Its has 54 percent sequence homology to previously reported KGF (FGF-7)[12]. FGF-10 and KGF has a high affinity to the same receptor, the KGFR isoform of FGFR 2 iiib, which is exclusively expressed by epithelial cells[26]. FGF-10 is known to have an important role in fetal development, particularly in limb and lung formation[27]. FGF-10 homozygous null mutant mouse (FGF-10 knockout mouse) embryos lack both forelimbs and hindlimbs, although a scapula is present at the correct forelimb position. These embryos have no lung buds at all, although presumptive trachea apparently develop normally[28].

FGF-10 accelerates skin wound healing, re-epithelialization and granulation tissue formation[29]. In a rabbit model of scar formation, FGF-10 is most effective in wound healing and causes no obvious scarring as compared with other growth factors, including KGF and TGF-beta which have previously been examined in this model[30]. Furthermore, recombinant FGF-10 stimulates prostatic epithelial cell growth[13].

In the FGF families, bFGF did not stimulate epithelial restitution of human colon mucosa in vitro[31]. In contrast, KGF has been reported to stimulate the growth of the mucin producing goblet cells in the colon[32]. Moreover KGF promoted the healing of colonic anastomoses in rats during a 1-week postoperative period following large bowel surgery[33].

The role of FGF-10 and its receptor in cancer tissues has not been elucidated well. Some cancer cells secrete fetal proteins and FGF-10 stimulate epithelial cell growth, suggesting that this growth factor may contribute to cancer cell growth. We previously reported that pancreatic cancer cells and colorectal cancer cells have a potential to synthesize KGF and may have a role in cancer cell growth in an autocrine and paracrine manner[21,24]. In our present study, FGF-10 and its receptor FGFR 2 iiib are colocalized in all cultured colorectal cancer cell lines examined. This finding suggests that FGF-10, usually produced by mesenchymal cells, is also synthesized by colorectal cancer cells. The existence of the FGFR 2 iiib receptor in colorectal cancer tissues may indicate the presence of an FGF-10-dependent paracrine and autocrine loop in the growth of this type of cancer. Recently we reported that a squamous carcinoma cell line expressed FGF-10 mRNA and recombinant FGF-10 stimulated growth of the cells[22]. To our knowledge, this is the first time that cancer cells classified as adenocarcinoma expressing FGF-10 mRNA and protein have been reported. Four forms of mouse FGF-10 protein with molecular weights from 20 to 30 kD have been reported. FGF-10 have two potential N-linked glycosylation sites and the 30 kD FGF-10 protein contains N-linked glycosylation[11]. A 30 kD FGF-10 protein is considered the major form of FGF-10 in human colorectal cancer cells, and the difference in molecular weight between cancer cell lines may be due to the glycosylation. To confirm the growth stimulatory effect of FGF-10 on colorectal cancer cells, we performed a growth assay using recombinant FGF-10. Administration of recombinant FGF-10 increased the rate of COLO 205 cell growth. These findings strongly indicate the important role of an FGF-10 in the growth of colorectal cancer cells. It is not clear why the maximal growth effect was exhibited on 1 ng/ml of FGF-10, but the concentration is possibly adequate for the growth of this type of cancer cell. In the present study, we did not

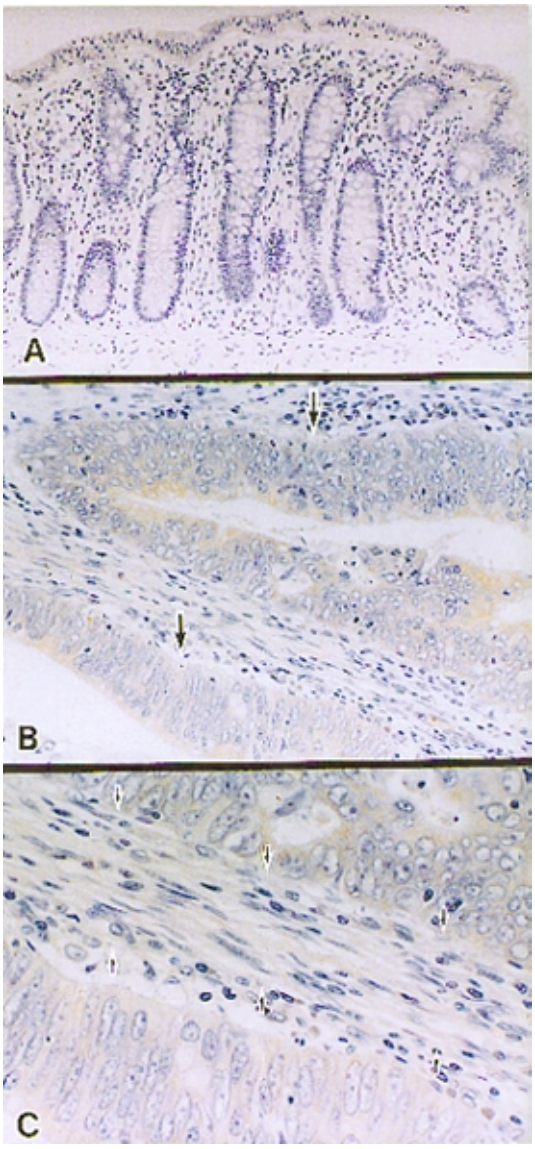

Fig. 4 Immunohistochemistry of FGF-10 in normal colorectal tissues and cancer tissues.
FGF-10 immunoreactivity is not observed in normal colorectal tissue (A), but localized in cancer cells (B, arrows), and fibroblasts adjacent to cancer cells (C, small arrows).
Original magnification: ×100 (A, B); ×400 (C).

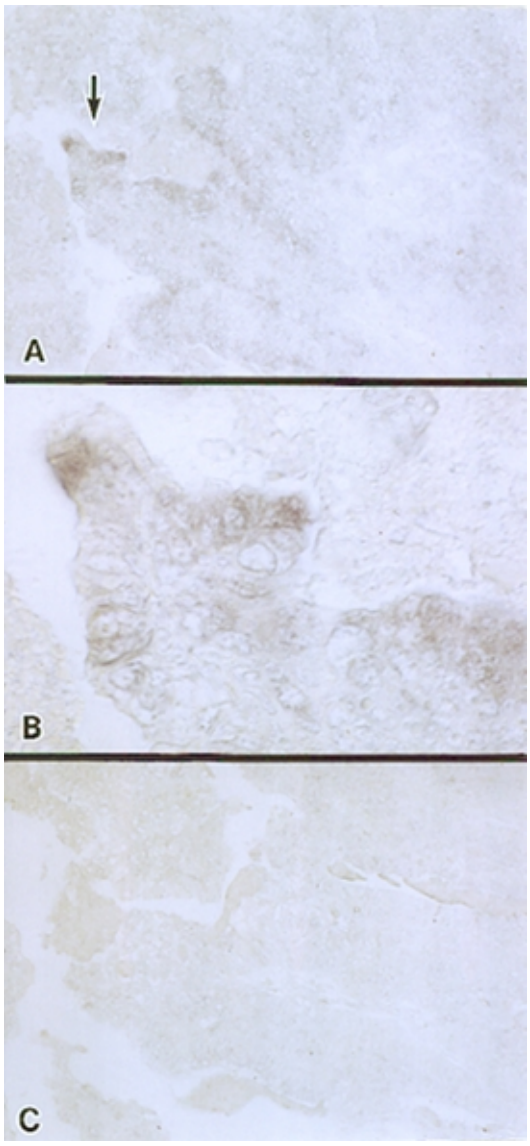

Fig. 5 In situ hybridization of FGF-10 mRNA in colorectal cancer tissues.
FGF-10 is expressed in adenocarcinoma cells (A arrows, B). In contrast, a sense FGF-10 probe does not show any signal (C).
Original magnification: ×60 (A, C); ×400 (B).

investigate the stimulatory effect of FGF-10 on other colorectal cancer cell lines, but these cancer cells also have FGFR 2 iiib, which suggests that FGF-10 may stimulate the growth of cells.

Our previous study demonstrated that FGFR 2 iiib (KGFR) mRNA was overexpressed in tissues from many human colorectal cancer cases[21]. FGF-10 and KGF are considered to share the same receptor, FGFR 2 iiib. KGF null mice show a disorder only in hair development, but FGF-10 null mice have lung and limb defects[34]. This finding may indicate the more important role of FGF-10 than KGF in epithelial cell growth. In colorectal cancer tissues, KGF was localized in cancer cells and enterochromaffin (EC) cells adjacent to cancer cells[21]. FGF-10 and its mRNA were localized mainly in cancer cells and fibroblasts adjacent to cancer cells, but they were not detected in EC cells. These findings indicate that FGF-10 is produced mainly by colorectal cancer cells and fibroblasts and that FGF-10 may act on cancer cell growth in an autocrine and paracrine manner via FGFR 2 iiib in col-

orectal cancer cases. Neutralizing antibodies for FGF-10 and FGFR 2 iiib could not be obtained, but we speculated that these antibodies may be effective for the inhibition of colorectal cancer cell growth.

The mechanisms that lead to overexpression of FGF-10 are not clear. Interestingly, TGF-beta and tumor necrosis factor-α (TNF-α) repressed FGF-10 mRNA expression, but EGF and interleukin-1-β (IL-1-β) had no such effect[11]. These factors are overexpressed in colorectal cancer tissues, so that different mechanisms for the regulation of FGF-10 may exist in colorectal cancer cells. Further study is necessary to investigate the signal transduction pathway for the induction of FGF-10 in colorectal cancer cells.

In summary, FGF-10 and FGFR 2 iiib were coexpressed in five cultured colorectal cancer cell lines and FGF-10 induced growth of COLO 205 colorectal cancer cells. Moreover, the expression of FGF-10 mRNA in colorectal cancer cells and adjacent fibroblasts may lead to cancer cell growth.

Acknowledgements: The authors thank Dr. Yue Ping Lu, Mr. Takenori Fujii and Ms Kiyoko Kawahara (Department of Pathology, Nippon Medical School) for their technical assistance and Professor Yukichi Moriyama (Center for Digestive Diseases, Second Affiliated Hospital of Nippon Medical School) for providing tissue samples and invaluable advice.

References

1. Mizuno S, Yamaguchi N, Watanabe S: Cancer mortality trends in Japan 1960－1990: Three-dimensional graphical presentation. Jpn J Clin Oncol 1992; 22: 433–436.
2. Shiraishi A, Ishiwata T, Shoji T, Asano G: Expression of PCNA, basic fibroblast growth factor, FGF-receptor and vascular endothelial growth factor in adenomas and carcinomas of human colon. Acta Histochem Cytochem 1995; 28: 21–28.
3. Picon A, Gold LI, Wang J, Cohen A, Friedman E: A subset of metastatic human colon cancers expresses elevated levels of transforming growth factor β 1. Cancer Epidemiol Biomark Prev 1998; 7: 497–504.
4. Daughaday W: The possible autocrine/paracrine and endocrine roles of insulin-like growth factors of human tumors. Endocrinol 1990; 127: 1–4.
5. Nabeshima K, Shimano Y, Inoue T, Itoh H, Kataoka H, Koono M: Hepatocyte growth factor/scatter factor induces not only scattering but also cohort migration of human colorectal adenocarcinoma cells. Int J Cancer 1998; 78: 750–759.
6. Fujita S, Sugano K: Expression of c-met proto-oncogene in primary colorectal cancer and liver metastases. Jpn J Clin Oncol 1997; 27: 378–383.
7. Price JE, Wolf JK, Pathak S: Distinctive karyotypes and growth patterns in nude mice reveal cross-contamination in an established human cancer cell lines. Oncology Rep 1998; 5: 261–266.
8. Remacle-Bonnet MM, Culouscou JM, Garrouste FL, Rabenandrasana C, Marvaldi JL, Pommier GJ: Expression type I, but not type II insulin-like growth factor receptor on both undifferentiated and differentiated HT 29 human colon carcinoma cell line. J Clin Endocrinol Metab 1992; 75: 609–616.
9. Yamasaki M, Miyake A, Tagashira S, Itoh N: Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family. J Biol Chem 1996; 271: 15918–15921.
10. Emoto H, Tagashira S, Mattei M-G, Yamasaki M, Hashimoto G, Katsumata T, Negoro T, Nakatsuka M, Birnbaum D, Coulier F, Itoh N: Structure and expression of human fibroblast growth factor-10. J Biol Chem 1997; 272: 23191–23194.
11. Beer H-D, Florence C, Dammeier J, Mcguire L, Werner S, Duan DR: Mouse fibroblast growth factor 10: cDNA cloning, protein characterization, and regulation of mRNA expression. Oncogene 1997; 15: 2211–2218.
12. Igarashi M, Finch PW, Aaroson A: Characterization of recombinant human fibroblast growth factor 10 reveals functional similarities with keratinocyte growth factor. J Biol Chem 1998; 273: 13230–13235.
13. Nakano K, Fukabori Y, Itoh N, Lu W, Kan M, Mckeehan WL, Yamanaka H: Androgen-stimulated human prostate epithelial growth mediated by stromal-derived fibroblast growth factor-10. Endocrine J 1999; 46: 405–413.
14. Smallwood PM, Munoz-Sanjuan I, Tong P, Macke JP, Hendry SHC, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J: Fibroblast growth factor(FGF) homologous factors: New members of the FGF family implicated in nervous system development. Proc Natl Acad Sci USA 1996; 93: 9850–9857.
15. McWhirter JR, Goulding M, Weiner JA, Chun J, Murre C: A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodoma in oncoprotein E 2 A-Pbx 1. Development 1997; 124: 3221–3232.
16. Miyake A, Konishi M, Martin FH, Hernday NA, Ozaki K, Yamamoto S, Mikami T, Arakawa Itoh N: Structure and expression of a novel member, FGF-16, of the fibroblast growth factor family. Biochem Biophys Res Com 1998; 243: 148–152.
17. Hoshikawa M, Ohbayashi N, Yonamine A, Konishi M, Ozaki K, Fukui S, Itoh N: Structure and expression of a novel fibroblast growth factor, FGF-17, preferentially expressed in the embryonic brain. Biochem Biophys Res Com 1998; 244: 187–191.
18. Hu MC-T, Qiu WR, Wang Y-P, Hill D, Ring BD, Scully S, Bolon B, DeRose M, Luethy R, Simonet WS, Arak-

awa T, Danilenko DM: FGF-18 a novel member of the fibroblast growth factor family, stimulates hepatic and intestinal proliferation. Mol Cell Biol 1998; 18: 6063–6074.
19. Nishimura T, Utsunomiya Y, Hoshikawa M, Hoshikawa M, Ohuchi H: Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. Biochem Biophys Acta 1999; 1444: 148–151.
20. Xie M-H, Holcomb I, Deuel B, Dowd P, Huang A, Vagts A, Foster J, Liang J, Brush J, Gu Q, Hillan K, Goddard A, Gurney AL: FGF-19, a novel fibroblast growth factor with unique specificity for FGFR 4. Cytokine 1999; 11: 729–735.
21. Watanabe M, Ishiwata T, Nishigai K, Moriyama Y, Asano G: Overexpression of keratinocyte growth factor in cancer cells and enterochromaffin cells in human colorectal cancer. Pathol Int 2000; 50: 363–372.
22. Kurban G, Ishiwata T, Lu Y-P, Fujii T, Kawahara K, Naito Z, Yamada N, Asano G: Expression and intracytoplasmic signal transduction pathway of fibroblast growth factor(FGF)-10 in human cervical cancer cell lines. J Nippon Med Sch 2001; 68: 253–258.
23. Lu Y-P, Nishigai K, Ishiwata T, Asano G: Differential expression of hepatocyte growth factor and its receptor(c-Met) in a rat artificial anus model. Wound Rep Reg 2000; 8: 59–67.
24. Ishiwata T, Friess H, Buchler MW, Lopez ME, Korc M: Characterization of keratinocyte growth factor and receptor expression in human pancreatic cancer. Am J Pathol 1998; 153: 213–222.
25. Ishiwata T, Kornmann M, Beger HG, Korc M: Enhanced fibroblast growth factor 5 expression in stromal and exocrine elements of the pancreas in choronic pancreatitis. Gut 1998; 43: 134–139.
26. Finch PW, Murphy F, Cardinale I, Krueger JG: Altered expression of keratinocyte growth factor and its receptor in psoriasis. Am J Pathol 1997; 151: 1619–1628.
27. Bellusci S, Grindley J, Emoto H, Itoh N, Hogan BLM: Fibroblast Growth Factor 10 and branching morphogenesis in the embryonic mouse lung. Development 1997; 124: 4867–4878.
28. Min H, Danilenko DM, Scully SA, Bolon B, Ring BD, Tarpley JE, DeRose M, Simonet WS: Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. Genes Dev 1998; 12: 3156–3161.
29. Xia Y-P, Zhao Y, Marcus J, Jimenez PA, Ruben SM, Moore PA, Khan F, Mustoe TA: Effects of keratinocyte growth factor-2 on wound healing in an ischaemia-impaired rabbit ear model and on scar formation. J Pathol 1999; 188: 431–438.
30. Wu L, Xia Y-P, Roth SI, Gruskin E, Mustoe TA: TGF-β 1 fails to stimulate wound healing and impairs its signal transduction in an aged ischemic ulcer model: importance of oxygen and age. Am J Pathol 1999; 154: 301–309.
31. Riegler M, Sedivy R, Sogukoglu T, Cosentini E, Bischof G, Teleky B, Feil W, Schiessel R, Hamilton G, Wenzl E: Effect of growth factors on epithelial restitution of human colonic mucosa in vitro. Scand J Gastroenterol 1997; 32: 925–932.
32. Housley RM, Morris CF, Boyle W, Ring B, Biltz R, Tarpley JE, Aukerman SL, Devine PL, Whitehead RH, Pierce GF: Keratinocyte growth factor induces proliferation of hepatocytes and epithelial cells throughout the rat gastrointestinal tract. J Clin Invest 1994; 94: 1764–1777.
33. Egger B, Tolmos J, Procaccino F, Sarosi I, Friess H, Buchler MW, Stamos M, Eysselein VE: Keratinocyte growth factor promotes healing of left-sided colon anastomoses. Am J Surg 1998; 176: 18–24.
34. Guo L, Degenstein L, Fuchs E: Keratinocyte growth factor is required for hair development but not for wound healing. Genes & Dev 1996; 10: 165–175.

(Received, February 22, 2001)
(Accepted, April 27, 2001)