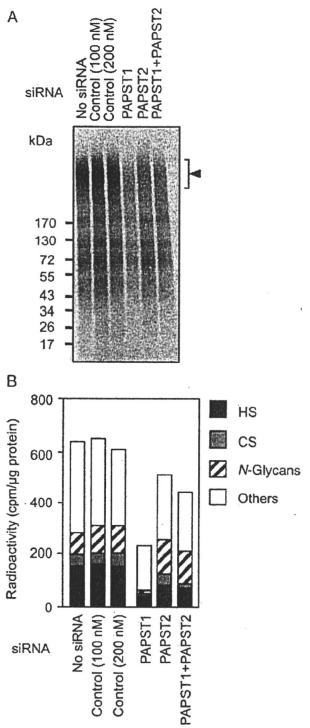

Fig. 5. Metabolic labeling of DLD-1 cells treated with siRNAs. DLD-1 cells were treated with various siRNAs on days 1, 4 and 7 and analyzed on day 10. (**A**) Autoradiograph of incorporated radioactivity into cellular proteins. Cells treated with each siRNA were labeled with $Na_2[^{35}S]O_4$ for 24 h and analyzed as described in Experimental procedures. Cellular proteins were separated by 2–15% gradient SDS–PAGE. The arrowhead indicates the signal that disappeared upon treatment with HSase. (**B**) Ratio of sulfate incorporation into HS, CS and *N*-glycans in cellular proteins. The amounts of incorporated sulfate into HS, CS and *N*-glycans were estimated on the basis of the radioactivity released after the treatment of cells or cellular proteins with HSase, CSase and PNGase F, respectively. The amount of total sulfate incorporation into proteins was determined by the precipitation of the proteins in the cell lysate with TCA. No siRNA, cells treated with no siRNA; control (100 nM), cells treated with 100 nM control siRNA; control (200 nM), cells treated with 200 nM control siRNA; PAPST1, cells treated with 100 nM *PAPST1* siRNA; PAPST2, cells treated with 100 nM *PAPST2* siRNA; PAPST1+PAPST2, cells treated with 100 nM *PAPST1* siRNA and 100 nM *PAPST2* siRNA.

On day 9 (i.e. 2 days after the third transfection), the cells were labeled with $Na_2[^{35}S]O_4$ for 24 h. Figure 5A shows the autoradiograph of the cellular proteins in the cell lysates.

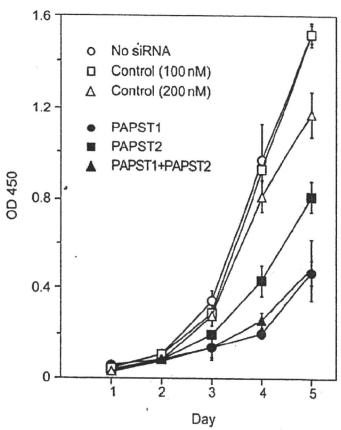

Fig. 6. Cellular proliferation of DLD-1 cells treated with siRNA. DLD-1 cells were transfected with each siRNA on days 1, 4 and 7 and seeded onto a 96-well plate on day 8. The number of cells was quantified once per day for 5 days using the WST-8 assay. No siRNA, cells treated with no siRNA; control (100 nM), cells treated with 100 nM control siRNA; control (200 nM), cells treated with 200 nM control siRNA; PAPST1, cells treated with 100 nM *PAPST1* siRNA; PAPST2, cells treated with 100 nM *PAPST2* siRNA; PAPST1+PAPST2, cells treated with 100 nM *PAPST1* siRNA and 100 nM *PAPST2* siRNA.

Treatment with either *PAPST1* or *PAPST2* siRNA reduced the density of HS (Figure 5A, arrowhead) and other signals. Figure 5B shows the sulfate incorporation into HS, CS, *N*-glycans and other glycans in the DLD-1 cells treated with each siRNA. The cells treated with *PAPST1* siRNA reduced the extent of sulfate incorporation into HS to one-third that of the cells treated with control siRNA. Treatment with *PAPST2* siRNA was less effective; the extent of sulfate incorporation into HS was half that of the cells treated with control siRNA. In the DLD-1 cells, treatment with *PAPST1* siRNA showed an effect on sulfation of HS, CS, and *N*-glycans, whereas treatment with *PAPST2* siRNA was mainly effective on HS (Figure 5B). These results indicate that gene expression of PAPS transporters regulates sulfation in this colorectal carcinoma cell line.

Gene silencing of PAPS transporters decreases cell growth in DLD-1 cells

Cellular proliferation of the siRNA-treated cells was further analyzed. On day 8 (i.e. 1 day after the third transfection), the siRNA-treated cells were seeded onto a 96-well plate and cell growth was measured once per day for 5 days using the WST-8 assay. Cells treated with either *PAPST1* or *PAPST2* siRNA showed significantly decreased cell growth relative to the cells treated with control siRNA (Figure 6). Similar to the result of sulfation, *PAPST1* siRNA was found to have a greater effect on cell growth than *PAPST2* siRNA in the DLD-1 cells.

The cell growth of double-knockdown cells was comparable to that of *PAPST1*-single knockdown cells. These results indicate that PAPS transporters play a role in the proliferation of colorectal carcinoma cells by controlling their sulfation status.

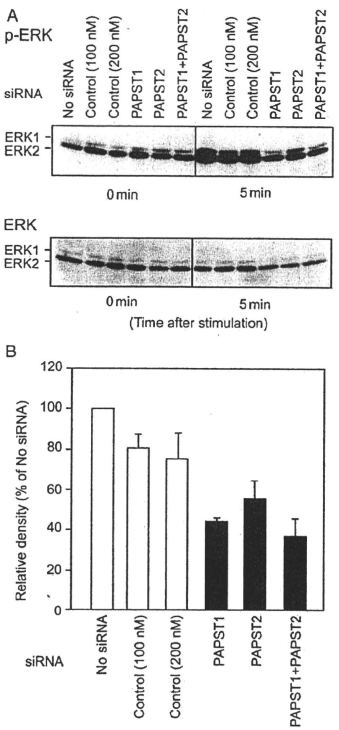

Fig. 7. FGF signaling of DLD-1 cells treated with siRNA. (**A**) Western blot analysis of ERK phosphorylation. DLD-1 cells were treated with each siRNA on days 1, 4 and 7 and stimulated with 10 ng/mL of FGF-2 on day 10. Cell lysates were prepared 0 and 5 min after stimulation and western blot analysis against ERK or phosphorylated ERK antibodies was performed. (**B**) Densitometric analysis of the western blot. Levels of phosphorylated ERK/total ERK 5 min after stimulation were calculated using the NIH Image program and the value obtained from control cells is presented as 100%. Values shown are means (SDs) obtained from three independent experiments. No siRNA, cells treated with no siRNA; control (100 nM), cells treated with 100 nM control siRNA; control (200 nM), cells treated with 200 nM control siRNA; PAPST1, cells treated with 100 nM *PAPST1* siRNA; PAPST2, cells treated with 100 nM *PAPST2* siRNA; PAPST1+PAPST2, cells treated with 100 nM *PAPST1* siRNA and 100 nM *PAPST2* siRNA.

Gene silencing of PAPS transporters reduces HS-dependent growth factor signaling in DLD-1 cells

It is known that HS is involved in many growth-factor signaling pathways with interacting growth factors. FGF-2 is one of the HS-interacting growth factors and plays a role in regulating the proliferation of cells. Because the interaction between HS and FGF-2 requires the sulfation of HS (Rapraeger et al. 1991; Yayon et al. 1991), FGF-2 signaling was analyzed in the siRNA-treated DLD-1 cells. For this experiment, the DLD-1 cells treated with each siRNA were stimulated with 10 ng/mL FGF-2 on day 10 (i.e. 3 day after the third transfection), and the transduction of FGF signaling was assessed in terms of the phosphorylation of extracellular signal-regulated kinase (ERK). Western blots of phosphorylated ERK and total ERK in the siRNA-treated cells are shown in Figure 7A. In cells treated with either *PAPST1* or *PAPST2* siRNA, the ratio of phosphorylated ERK/total ERK was decreased relative to the cells treated with control siRNA (Figure 7B). The double-knockdown cells showed the lowest value for the ERK phosphorylation. The transduction of FGF signaling reflected the sulfation status of HS in the siRNA-treated DLD-1 cells. These results indicate that the expression of PAPS transporter genes affects HS-dependent growth factor signaling in colorectal carcinoma cells.

Discussion

The present study showed that the expression level of *PAPST1* is several times higher than that of *PAPST2* in colorectal carcinoma cell lines (Figure 1A). In colorectal carcinoma tissues, the difference in expression levels of two PAPS transporters was less prominent than the difference in the cell lines (Figure 1B). Immunohistochemical analyses revealed that PAPST1 protein is predominantly expressed in epithelial cells in both noncancerous and cancerous colorectal tissues (Figure 2E–I). The expression of PAPST1 was found to be remarkably increased in fibroblasts around invasive cancer cells (Figure 2I, asterisks) but did not change in epithelial carcinoma cells of cancerous colorectal tissues. In contrast, PAPST2 protein was strongly detected in epithelial cells in noncancerous colorectal tissues (Figure 2J), whereas the expression was faintly detectable in epithelial cells in cancerous colorectal tissues (Figure 2K). Therefore, the difference in the expression levels of PAPS transporters in colorectal carcinoma cell lines might be associated with decreased *PAPST2* expression in epithelial carcinoma cells.

Several studies have reported that the composition of sulfated glycoconjugates is altered in colorectal tissues during carcinogenesis. The sialyl 6-sulfo Le^x (Izawa et al. 2000) and 3′-sulfo-Le^a (Matsushita et al. 1995; Yamachika et al. 1997) epitopes are predominantly expressed in nonmalignant colorectal tissues, but are not detected in the malignant tissues. The impaired syntheses of sialyl 6-sulfo Le^x and disialyl Le^a upon malignant transformation are responsible for the accumulation of sialyl Le^x and sialyl Le^a in colon cancer cells (Izawa et al. 2000; Kannagi 2004; Miyazaki et al. 2004). Very recently, Yusa et al. (2010) reported that diminished transcription of sulfate transporter gene *DTDST* causes decreased expression of sialyl 6-sulfo Le^x and increased expression of sialyl Le^x in colon cancer cells. Sulfate transporters and PAPS synthases are

involved in PAPS synthesis, whereas PAPS transporters are required for sulfation reaction in the Golgi apparatus. The present study showed that the expression status of PAPS transporters is also a key factor in sulfation of cellular proteins in colon cancer cells. In addition, it has been shown that several sulfotransferases exhibit altered expression levels and activities in colorectal carcinomas (Vavasseur et al. 1994; Yang et al. 1994; Kuhns et al. 1995; Seko et al. 2002a, 2002b). Further identification of alterations of sulfated glycoconjugates and components involved in sulfation during malignancy will provide valuable insights into the role of sulfation in cancer.

Silencing of PAPS transporter genes influences sulfation of proteoglycans and proliferation of colorectal carcinoma cells. Dick et al. (2008) reported that the overexpression of PAPST1 enhances the sulfation of CS in the apical pathway of MDCK cells but does not affect HS sulfation. In contrast, the present work indicates that silencing of *PAPST1* gene expression in DLD-1 cells results in a decrease in both HS and CS sulfation (Figure 5). It is known that sulfotransferases for CS have higher K_m values for PAPS than the sulfotransferases for HS (Kolset et al. 2004). The results of the present study indicate that the sulfotransferases for HS are also susceptible to the levels of substrate in the Golgi apparatus in colorectal carcinoma cells.

Silencing of PAPS transporters reduces FGF-2 signaling in DLD-1 cells. It has been reported that both HS (Rapraeger et al. 1991; Yayon et al. 1991) and CS (Deepa et al. 2002) bind to FGF-2. Because the amount of cell surface HS was found to be more than three times greater than that of cell surface CS (Figure 3), HS is considered to contribute mainly to FGF-2 signaling in DLD-1 cells. Numerous studies have shown that cell surface HS plays a crucial role in normal growth and development. It is well known that HS is required for regulation of many growth factor-signaling pathways, such as FGF (Rapraeger et al. 1991; Yayon et al. 1991), wingless/Wnt (Reichsman et al. 1996), heparin-binding, epidermal growth factor-like growth factor (Aviezer and Yayon 1994), hepatocyte growth factor (Zioncheck et al. 1995) and vascular endothelial growth factor (Soker et al. 1994; Tessler et al. 1994). We previously demonstrated that PAPS transporters are essential for normal development in *Drosophila* (Kamiyama et al. 2003; Kamiyama and Nishihara 2004; Goda et al. 2006). Lüders et al. (2003) also demonstrated that the *Drosophila* ortholog of *PAPST1* is required for signaling of wingless and hedgehog. Therefore, alteration of HS sulfation may be a significant regulatory factor for cellular proliferation in colorectal carcinomas, although the significance of CS and other glycans should also be considered. Additionally, the strong expression of PAPST1 protein in fibroblasts in the vicinity of invasive cancer cells (Figure 2I, asterisks) suggests that PAPST1 has a role in the desmoplastic reaction during tumorigenesis to support cancer growth through modulation of HS-dependent signaling.

It has been reported that the expression of nucleotide sugar transporters is altered in the case of cancer or inflammation. In these studies, it was found that the expression level of a UDP-galactose transporter (SLC35A2) is increased in human colon carcinoma and is responsible for the synthesis of Thomsen–Friedenreich antigens and sialyl Le^a and sialyl Le^x epitopes (Kumamoto et al. 2001). Expression of a GDP-fucose transporter (SLC35C1) is upregulated in hepatocellular carcinomas and plays a role in increased fucosylation (Moriwaki et al. 2007). In addition, the expression levels of transporters involved in sulfo sialyl Le^x glycan biosynthesis were coordinately upregulated by inflammation-related stimuli (Huopaniemi et al. 2004). Koike et al. (2004) reported that transcription of genes involved in the synthesis of the E-selectin ligands, namely fucosyltransferase VII, sialyltransferase ST3Gal-I and UDP-galactose transporter 1 (SLC35A2), is significantly induced in cancer cells under hypoxic culture conditions. Their study also showed that a hypoxia-inducible transcription factor induces transcription of these genes and leads to a significant increase in selectin-mediated cancer cell adhesion to endothelial cells (Koike et al. 2004). Furthermore, Yusa et al. (2010) suggested that the transcription of sulfate transporter *DTDST* is suppressed by epigenetic silencing via histone modification in colon cancer cells. It would be an interesting topic of research to elucidate how the expression of these transporter genes is transcriptionally regulated in cancer or inflammation.

Mutations in nucleotide sugar transporter genes responsible for several disorders have been recently identified (Lübke et al. 2001; Lühn et al. 2001; Martinez-Duncker et al. 2005; Hiraoka et al. 2007). With regard to the synthesis of GAGs, the mutation of a gene involved in the synthesis of CS, *SLC35D1*, was reported to be responsible for Schneckenbecken dysplasia, a severe skeletal dysplasia (Hiraoka et al. 2007). A missense mutation in the bovine *SLC35A3* gene, which encodes a UDP-*N*-acetylglucosamine transporter, causes complex vertebral malformation (Thomsen et al. 2006). To date, no disorders associated with PAPS transporter gene mutation have been reported. However, Clement et al. (2008) recently demonstrated that a zebrafish with a *PAPST1* mutation has cartilage defects that strongly resemble those seen in human patients with hereditary multiple exostoses. Additionally, analyses of *Drosophila* mutants demonstrated the significance of PAPS transporters in development (Kamiyama et al. 2003; Lüders et al. 2003; Goda et al. 2006). Our recent research has revealed that both PAPST1 and PAPST2 contribute to the maintenance and differentiation of mouse embryonic stem cells by regulating Wnt, bone morphogenetic protein and FGF signaling (Sasaki et al. 2009). The results of the present study provide additional information on the functions of PAPS transporters in cancer cells. In the future, approaches using transgenic or knockout mice would be helpful in elucidating the key roles of PAPS transporters.

Experimental procedures

Cell culture and tissue samples

Omega, DLD-1 and LS174T cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1) medium (Invitrogen Co., Carlsbad, CA), supplemented with 10% fetal bovine serum and 1× penicillin/streptomycin (Invitrogen).

The use of the clinical materials was approved by the ethical committee of the National Hospital Organization Osaka National Hospital, the Keio University Hospital, the

National Institute of Advanced Industrial Science and Technology, the Aichi Cancer Center Research Institute and Soka University. The formalin-fixed and paraffin-embedded colon cancer samples were obtained from the National Hospital Organization Osaka National Hospital and used for immunohistochemical analysis. The frozen rectal cancer samples were obtained from the Keio University Hospital and used for *in situ* hybridization. The frozen cancerous and non-cancerous colon tissues were obtained from the National Hospital Organization Osaka National Hospital and the Aichi Cancer Center Research Institute to use for quantitative PCR analysis.

Quantitative analysis of PAPST1 and PAPST2 transcripts

The quantities of *PAPST1* and *PAPST2* transcripts were determined using real-time PCR. Total RNA from human colorectal tissues was extracted using RNeasy Plus Mini (QIAGEN K.K., Tokyo, Japan) or the method of Chomczynski and Sacchi (1987). The features of each of the colorectal carcinoma samples were confirmed by histopathological examination (Table I). Total RNA from the cell lines was prepared using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized with a Superscript II First Strand Synthesis kit (Invitrogen) with an oligo-dT primer. Real-time PCR was performed using qPCR Mastermix (QuickGoldStar; Eurogentec, Seraing, Belgium) and an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster, CA).

The PCR primer pair sequences and TaqMan probes used for each gene were same as those previously reported (Kamiyama et al. 2003, 2006). The relative amounts of *PAPST1* and *PAPST2* transcripts were normalized to the amount of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) transcript present in the same cDNA.

In situ hybridization

A pBluescript SK(−) plasmid containing a 0.57 kb *PAPST1* sequence was linearized with *Not*I. A digoxigenin-labeled antisense riboprobe was synthesized with T7 RNA polymerase using Dig RNA Labeling Kits (Roche Applied Science, Indianapolis, IN). As a negative control, a sense riboprobe was synthesized with T3 RNA polymerase after linearization of the plasmid with *Xho*I.

Serial frozen sections prepared from rectal tissues in Tissue-Tech OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan) were thawed on slides and the OCT compound was removed. Sections were treated with 1 μg/mL of proteinase K at 37°C for 10 min and refixed in 4% paraformaldehyde at 4°C for 20 min. Sections were then pre-hybridized in hybridization buffer (5× SSC containing 50% deionized formamide, 10% dextran sulfate, 0.5% Tween 20, 5 mM dithiothreitol, 50 μg/mL of heparin and 50 μg/mL of yeast tRNA) and hybridized with a digoxigenin-labeled sense or antisense riboprobe in hybridization buffer at 58°C for 16 h. After hybridization, sections were sequentially washed with 5× SSC containing 50% formamide and 0.2% Tween-20 at 58°C for 30 min and three times with 2× SSC containing 50% formamide and 0.2% Tween-20 at 58°C for 30 min. The sections were then treated with 0.5% blocking buffer (Roche Applied Science) in TBST and reacted with alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche Applied Science). Endogenous phosphatases were inactivated with 2 mM levamisole in TBST and riboprobes were detected with 0.375 mg/mL of nitroblue tetrazolium, 0.175 mg/mL of 5-bromo-4-chloro-3-indolyl phosphate, 100 mM NaCl, 100 mM Tris–HCl, pH 9.5, 50 mM $MgCl_2$ and 0.1% Tween-20 for 2.5 h. The developed sections were washed three times with 10 mM Tris–HCl, pH 8.0, and 1 mM EDTA and were mounted with glycerol.

Immunohistochemical analysis

Rabbit polyclonal antibodies generated against mouse PAPST1, KAVPTEPPVQKV, and human PAPST2, CAKNPVRTYGYA, were purified by using each peptide and were then used to examine the immunohistochemical distribution of PAPS transporter proteins.

For immunostaining of formalin-fixed and paraffin-embedded tissue samples, 3 μm thick sections were serially cut and mounted on precoated slides. A Ventana system (Ventana XT system BenchMark; Ventana Medical Systems, Tucson, AZ) was used for immunohistochemical analysis. All procedures were performed automatically by the system according to the manufacturer's protocols. Briefly, the tissue sections were automatically treated with an antigen-retrieval solution (Ventana) and heated on a slide heater at 100°C for 30 min. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide for 4 min. The sections were then incubated with anti-PAPST1 antibody (rabbit polyclonal, 1/500 dilution) for 30 min at 37°C. Detection was performed using the LSAB Ventana Iview DAB detection system according to the manufacturer's instructions. Sections were counterstained with hematoxylin.

For immunostaining of frozen tissue sections, the sections were fixed in cold acetone for 5 min and then rehydrated in phosphate-buffered saline (PBS). The sections were incubated with a blocking reagent (5% bovine serum albumin in PBS) at room temperature for 30 min and reacted with the anti-PAPST1 antibody (rabbit polyclonal, 1:500 dilution) or the anti-PAPST2 antibody (rabbit polyclonal, 1:100 dilution) at 4°C for 16 h. After washing with PBS, the sections were incubated with anti-rabbit immunoglobulin (IgG)-conjugated Alexa Fluor 488 at room temperature for 30 min. For counter staining, the sections were treated with phallotoxins conjugated Alexa Fluor 594 (Molecular Probes, Invitrogen, Eugene, OR) to stain the F-actin for 10 min and then incubated with Hoechst 33342 to stain the nucleus for 10 min. The sections were mounted in ProLong Gold Antifade reagent (Molecular Probes).

Verification of antibody specificity

The immunoreactivity of purified anti-PAPST1 antibody to human PAPST1 protein was confirmed by western blotting. The coding region of human PAPST1 was amplified by PCR using a forward primer 5′-GAATTCTGGACGCCAGATGG TGG-3′ and a reverse primer 5′-CTCGAGTCAAACCTT CTGCACAGGAG-3′. The PCR fragment was subcloned into the *EcoR*I and *Xho*I sites of the pCXN2-c-myc vector which contains an N-terminal-c-myc tag. HEK 293 cells were subcultured onto 6 cm dishes at a concentration of 1×10^6 cells/

dish and were transfected with 2 μg of plasmid using Lipofectamine 2000 reagent (Invitrogen). Three days after the transfection, cells were suspended in 120 μL of 10 mM triethanolamine containing 0.8 M Sorbitol and lysed with 60 μL of 3× SDS sample buffer (New England Biolabs Inc., Ipswich, MA) at 4°C for 16 h. The whole cell lysate (1 μg protein) was subjected to 10% SDS–polyacrylamide gel electrophoresis (PAGE), and proteins were transferred onto polyvinylidene difluoride membranes (Millipore). The membrane blot was blocked with 5% skimmed milk in PBS containing 0.1% Tween-20 (PBST, pH 7.4) and then was immunoreacted with the anti-PAPST1 antibody (1:5000 dilution in PBST) or an anti-c-myc monoclonal antibody (1:5000 dilution in PBST; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). After washing with PBST, each blot was reacted with the corresponding secondary antibody conjugated with horseradish peroxidase in PBST. The blot was washed with PBST and developed with ECL Plus reagents (GE Healthcare Bioscience, Piscataway, NJ).

Metabolic labeling and determination of total sulfate incorporation into proteins

Twenty-four hours prior to analysis, cells were subcultured in a 24-well plate at a concentration of 1×10^5 cells/well in inorganic sulfate-free DMEM/F12 medium supplemented with 10% fetal bovine serum and 100 μCi/mL of carrier-free $Na_2[^{35}S]O_4$ (American Radiolabeled Chemicals Inc., St. Louis, MO). The cells were rinsed twice with PBS, suspended in 50 μL of lysis buffer (10 mM Tris–HCl, pH 7.4, 0.5% Nonidet P-40, 1 mM EDTA and 0.5 mM phenylmethylsulfonyl fluoride) and incubated on ice for 1 h. The solution was centrifuged at 18,500 × *g* for 30 min, and the supernatant was used as the cell lysates. Twenty micrograms of the protein in each sample was precipitated with 10% TCA and washed with 5% TCA, followed by cold acetone. The precipitate was dried and dissolved in 50 μL of 0.5 N NaOH for scintillation counting.

Determination of sulfate incorporation into HS, CS and N-linked glycans

The $Na_2[^{35}S]O_4$-labeled cells were rinsed twice with PBS and cultured in normal medium for 2 h. Cells were rinsed with PBS and treated with 0.5 mL of DMEM/F12 medium containing 10 mU/mL of heparitinase (Seikagaku Kogyo, Tokyo, Japan) or 100 mU/mL of chondroitinase ABC (Seikagaku Kogyo) at 37°C for 2 h. The medium was saved and centrifuged at 18,500 × *g* for 5 min, and the supernatant was used for scintillation counting. For each sample, the value was calculated as the difference between the radioactivity obtained from cells treated with the enzyme and the background radioactivity without the enzyme.

For quantification of sulfate incorporation into *N*-linked glycans, 20 μg of protein from the cell lysate was treated with PNGase F (New England BioLabs) at 37°C for 2 h. Proteins were precipitated with 20% TCA and washed with 5% TCA, followed by cold acetone. The precipitate was dried and dissolved in 50 μL of 0.5 N NaOH for scintillation counting. The value was calculated as the difference between the radioactivity obtained from the cell lysate without the enzyme and the radioactivity from the cell lysate treated with the enzyme.

Treatment of DLD-1 cells with siRNA

Nineteen-base pair siRNAs with two bases of 3′ overhangs were designed using the siDirect program (http://genomics.jp/sidirect). The siRNA sequences used were as follows: for control siRNA, sense strand 5′-GUACCGCACGUCAUUCG UAUC-3′ and antisense strand 5′-UACGAAUGACGUGC GGUACGU-3′; for PAPST1 siRNA, sense strand 5′-GGU CAAGAGAGCAUAGGUAGG-3′ and antisense strand 5′-UA CCUAUGCUCUCUUGACCCC-3′; for PAPST2 siRNA, sense strand 5′-CCAGUUCGGACCUAUGGUUAU-3′ and antisense strand 5′-AACCAUAGGUCCGAACUGGAU-3′.

DLD-1 cells were subcultured onto 6 cm dishes at a concentration of 1×10^6 cells/dish 24 h prior to transfection. The cells were repeatedly transfected with 100 nM siRNA with Lipofectamine 2000 reagent three times on days 1, 4 and 7. On day 9, the cells were labeled with $Na_2[^{35}S]O_4$ for 24 h and analyzed as described in 'Metabolic labeling and determination of total sulfate incorporation into proteins'. RNA was extracted using TRIzol reagent (Invitrogen) on day 10.

Cell proliferation assay

Cell proliferation was assessed using an assay with a tetrazolium salt, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium monosodium salt (WST-8). In this experiment, cells were seeded onto a 96-well plate at a concentration of 2×10^3 cells/well in quadruplicate on day 8 and cultured in the normal medium. The number of cells was quantified once per day for 5 days using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan). The absorbance at 450 nm was measured 2 h after the reaction using a microplate reader (Model 3550; Bio-Rad Labs, Hercules, CA). The obtained value was adjusted by subtracting the background value (obtained without the reagent).

Cell stimulation and western blot analysis

For activation of FGF signaling, cells were serum-starved for 16 h and treated with 10 ng/mL of FGF-2 (Upstate Biotechnology Inc., Lake Placid, NY) for 5 min. Cells were rinsed with ice-cold PBS and lysed in lysis buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl, 1% Triton X-100, 1 mM Na_3VO_4, 10 mM NaF and protease inhibitors) and centrifuged at 18,500 × *g* for 5 min. The supernatant was used for western blot analysis.

For western blot analysis, proteins in the cell lysate (5 μg) were separated with 10% SDS–PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). The membrane blot was blocked with 1% bovine serum albumin in 20 mM Tris-buffered saline containing 0.1% Tween-20 (TBST, pH 7.4) for 2 h at room temperature and then immunoreacted with an antibody against ERK-1/2 (Cell Signaling Technology, Beverly, MA) or phosphorylated ERK-1/2 (Thr-202 and Thr-204; Cell Signaling Technology) in blocking buffer at 4°C overnight. After washing with TBST, each blot was reacted with its corresponding secondary antibody conjugated with

horseradish peroxidase in TBST at room temperature for 1 h. The blot was washed with TBST and developed with ECL Plus reagents (GE Healthcare Bioscience).

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Conflict of interest statement

None declared.

Abbreviations

CS, chondroitin sulfate; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular signal-regulated kinase; FGF, fibroblast growth factor; GAG, glycosaminoglycan; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GDP, guanidine diphosphate; HS, heparan sulfate; IgG, immunoglobulin G; Le^a, galactose β 1-3[fucose α 1-4] *N*-acetylglucosamine; Le^x, galactose β 1-4[fucose α 1-3] *N*-acetylglucosamine; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PBST, PBS containing 0.1% Tween-20; PNGase, peptide:*N*-glycosidase; RNAi, RNA interference; siRNA, small interfering RNA; TBST, Tris-buffered saline containing 0.1% Tween-20; TCA, trichloroacetic acid; UDP, uridine diphosphate.

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肝・胆道系症候群(第2版)

—その他の肝・胆道系疾患を含めて—

II
肝臓編(下)

XIV　肝細胞癌以外の肝腫瘍

異所性肝と異所性肝細胞癌

森田香織
葛下典由
三田英治
中森正二

XIV 肝細胞癌以外の肝腫瘍

異所性肝と異所性肝細胞癌

Ectopic liver and ectopic hepatocellular carcinoma

Key words：異所性肝細胞癌，異所性肝

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1. 概念・定義

肝実質と連絡がない部位において，異所性に肝臓組織を認めるものを異所性肝という．Collanらにより，肝組織の位置異常を肝臓との連絡の有無および大きさにて①～④の4型に分類する方法が考案されている[1]．①accessory liver lobesと②small accessory liver lobesは，本来の肝臓と実質的連絡があるものを指し，茎を介してつながっている前者と，主に重量が10-30 gで肝被膜の外側に付着している後者に細分される．これらは肝副葉とされ，厳密な意味での異所性肝と区別している．一方，③ectopic liverは肝臓と実質的連絡のない肝組織を指し，一般的に‘異所性肝’と呼ばれているものは，このことを指す．通常，1-2 cmで手術や剖検時に偶然発見されることが多い．④microscopic ectopic liver tissueは，胆嚢壁内に迷入した肝組織で，顕微鏡で偶然発見されることが多い．この③+④を広義の異所性肝としている．本稿はこれに従う．異所性肝細胞癌とは，異所性肝組織より発生した肝細胞癌をいう．

2. 疫　　学

解剖学的に肝臓の発生過程で異常が生じることはまれとされてきた．しかし異所性肝は，最近の画像診断の進歩により，偶然発見され報告されている．我が国での頻度は，Watanabeらは腹腔鏡下の観察で1,060例中5例(0.47％)[2]，柴山らは0.86％と報告している[3]．発生部位は，圧倒的に胆嚢が多く，その他，大網，副腎，膵静脈索，胸腔，脾，胎盤などが報告されている．

異所性肝細胞癌の報告は極めて少なく，1969年の堀内らの報告[4]から2009年の著者らの報告[5]までで39例のみである．

異所性肝細胞癌は**表1**に示すように，我が国からの報告が39例中29例と多く，性別では男性が32例(82％)と圧倒的に多いのが特徴である．また，発生部位としては，異所性肝と異なりその分布は胆嚢などの肝臓周囲には偏ってい

表1　異所性肝細胞癌の特徴(文献[5]より改変)

項　目	特　徴
性差	男性32/39(82％)，女性7/39(18％)
平均年齢	62.1歳(34－81歳)
部位	腹部(32/39，82％)，胸壁・胸腔(7/39，18％)
大きさ	1-15 cm，5 cm以上が26/36(72％)
肝炎ウイルスマーカー	6/31(19％)が陽性
肝病態	肝硬変・慢性肝炎が13/36(36％)
αFP値(AFP)	24/31(77％)で上昇

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ないが大半を腹部に認めている．通常の肝細胞癌とは異なり，ウイルス性肝炎を基礎疾患としてもたない症例も少なくない．

3. 病因・病態

肝臓の発生は複雑で，胎齢18日，4mmの胎芽の頃に前腸と卵黄腸管の合流部付近に肝および胆道系の原基が認められ，胎齢25日頃に肝憩室が形成され肝臓部と胆嚢部にわかれる．肝臓部位は肝実質，肝内脈管網，肝管および胆嚢管が形成される[6]．異所性肝とは，このような発生の過程で異常が生じることで形成されるもので，Cullenによれば，1767年Morgagniによるものが最初の報告である[7]．我が国では1904年の田崎の報告が最初のものとされている[8]．分布は83％が肝周囲であり，前述のように，特に胆嚢周囲が65％と最も多い[9]．動脈，静脈，胆管をもつとされているが，病理学的に完全な小葉構造をもつものは少なく，胆汁の産生および排泄経路については不明であり，恐らく異所性肝と正常の排泄経路のどこかが細い胆管で交通し，異所性肝で産生された胆汁はその胆管を通り排泄されるものと推測されているが，組織学的に証明された例はない[10]．

異所性肝組織からの発癌については，肝細胞癌と異なり，ウイルス性肝炎や肝硬変を伴わない場合も少なくはなく，この理由について異所性肝組織は肝臓としての機能を備えていてもその機能が未熟で，肝炎ウイルス感染や，肝炎ウイルスマーカーが陰性で慢性肝障害がなかったとしても，肝細胞にて代謝される化学物質などの障害で発癌しやすいのではないかと考えられている[11]．

実際には**表1**に示すように，1969−2009年までの39例の報告の中で，肝炎ウイルスマーカーについて記載のあった31例中，陽性であったのは6例(19％)だけであった[5]．しかし，肝病態について記載のあった36例中13例(36％)が，肝硬変あるいは慢性肝炎と診断されており，当時はC型肝炎などの肝炎ウイルスマーカーについての測定系が不十分であったことも考慮すると，異所性肝細胞癌と，ウイルス性慢性肝炎・肝病態との関連は少なからずあると思われる．また，異所性肝組織の病変として，肝細胞癌のほかに海綿状血管腫[12]，神経芽細胞腫の転移[13]などの報告がある．

4. 診断と鑑別診断

異所性肝は，特に症状がなければ診断される機会はほとんどない．報告症例での発見契機は，胸部レントゲン異常陰影，strangulationによる心窩部痛の出現，腫瘤触知にて診断された例もあるが，ほとんどは手術，剖検，腹腔鏡検査時に偶然発見されている．

一方，異所性肝細胞癌は，破裂などによる腹腔内出血または腹痛などの症状で診断されていることが多い．肝臓から離れた部位にあり，また特徴的な画像所見などもないため，画像診断のみで確定診断を行うことは困難である．血液検査にてAFP高値であることが多いが，鑑別診断として肝臓原発の肝細胞癌およびAFP産生性腫瘍，卵巣腫瘍などが上げられるため，AFP-L3分画の測定も必要となる．

腫瘍の大きさについては，異所性肝の場合は2cm以下が約半数を占めており，10cm以上のものは一般的にみられない．一方，異所性肝細胞癌は最小1cmくらいから，最大15cmくらいまで様々であるが，5cm以上が36例中26例(72％)と多く[5]，このことが初診時の臨床症状と関連しているものと考えられる．

著者らが経験した症例のように，恥骨に骨転移をきたしていても原発巣は小さく，原発巣精査にCT，MRCP，PETなどの各種画像検索が必要であり，最終的には腹腔鏡検査，開腹術が必要とされる例もある(**図1-a，b，c**)[5]．また，有茎性肝細胞癌では，病理解剖学的に肝臓と実質的な連絡はないが栄養血管が肝臓動脈分枝より連絡されている場合がある．この場合は血管造影での検索が必要となる．最終的な確定診断は，やはり生検や手術などで得られた病理標本によってなされている．

5. 治療法と予後

異所性肝は，症状がなければ，原則的に治療

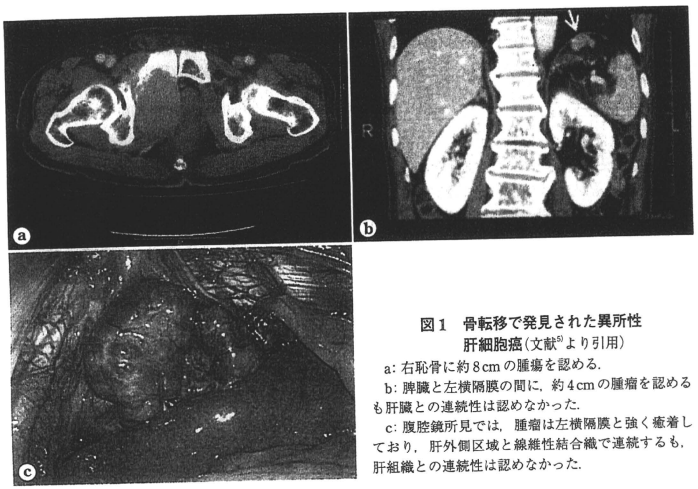

図1　骨転移で発見された異所性肝細胞癌（文献[5]より引用）
a: 右恥骨に約8cmの腫瘍を認める．
b: 脾臓と左横隔膜の間に，約4cmの腫瘤を認めるも肝臓との連続性は認めなかった．
c: 腹腔鏡所見では，腫瘤は左横隔膜と強く癒着しており，肝外側区域と線維性結合織で連続するも，肝組織との連続性は認めなかった．

の必要はないと考えられる．異所性肝細胞癌については，確立された治療法はない．単発例については手術で摘出し，腹膜播種や転移巣については化学療法を施行しているとの報告例もあるが，異所性肝細胞癌に対する標準的な化学療法は，症例数が少ないこともあって，現在確立されていない．異所性肝細胞癌の予後については，治療後の詳細な報告はみられないが，初診時に腫瘍が比較的大きく，また腹腔内に転移を伴うことも多いため，予後はよくないと推測される．また，病変が小さく完全切除が可能であっても，異所性肝細胞癌摘出3年後，肝臓内に肝細胞癌が出現した例も報告されており[14]，手術で摘出した症例についても注意深い経過観察が必要と考えられる．

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XIV

局所治療として減量再肝切除を施行した肝細胞癌症例

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Re-Resection of the Liver as Reduction Surgery for Hepatocellular Carcinoma: Atsushi Miyamoto, Shoji Nakamori, Masanori Tsujie, Masayoshi Yasui, Masakazu Ikenaga, Michihiko Miyazaki, Motohiro Hirao, Kazumasa Fujitani, Hideyuki Mishima and Toshimasa Tsujinaka (*Dept. of Surgery, National Hospital Organization, Osaka National Hospital*)

Summary

In this study, we report four cases of re-resection of the liver as reduction surgery for a failure of transcatheter hepatic arterial embolization (TAE) for multiple intrahepatic recurrences after hepatic resection. In all of the 4 cases, a liver function was well preserved and portal vein thrombosis was not revealed in preoperative CT scan. Although bile leakage was observed in one case, no major complications were observed in other 3 cases and their hospital stay after surgery was within 13 days. To date, one year survival rate after re-resection is 100%, and a short-term prognosis is good. On the basis of the result, re-resection of the liver as reduction surgery could be considered as one of options of the locoresional treatment for a failure of TAE for multiple intrahepatic recurrences. **Key words:** Hepatocellular carcinoma, Reduction surgery, Re-resection of the liver

要旨 肝細胞癌根治的切除の残肝多発再発に対する治療としてはTAEが選択されることが多いが，繰り返し施行しているうちに十分な効果が得られない無効病巣となり，治療に難渋することも少なくない。今回，われわれは初回肝切除後の残肝多発再発に対して繰り返しTAEを行い，TAE無効病巣が出現した4例に対して減量再肝切除を施行した。全例肝予備能はChild Aであり，画像検査で門脈内腫瘍栓は認めなかった。1例で術後胆汁漏を認め入院期間の延長を来したが，残りの3例では術後2週間以内に退院可能であった。現時点での1年生存率は100%であり，短期成績は良好であった。以上のことから，肝予備能が良好で門脈内腫瘍栓を認めない症例では，減量再肝切除はTAE無効病巣に対する局所治療の選択肢となり得ると考えられる。

はじめに

肝細胞癌根治的切除の残肝多発再発に対する治療としてはTAEが選択されることが多いが，繰り返し施行しているうちに十分な効果が得られない無効病巣となり，治療に難渋することも少なくない。

今回，われわれは初回肝切除後の残肝多発再発に対して繰り返しTAEを行い，TAE無効病巣に対して減量再肝切除を施行した4例について，その安全性と効果について検討した。

I. 症 例

症例1: 78歳，女性。S8部分切除後8か月目に再発を来し，以後TAEを9回，TAIを3回施行されたが，下横隔動脈から供血されるTAE無効病巣が出現した（図1a,b）。

症例2: 75歳，男性。S5/6部分切除後7か月目に再発を来し，以後TAEを6回施行されたが，下横隔動脈および大網から供血される病変がTAE無効病巣となった（図2a,b）。

症例3: 70歳，男性。後区域切除後6か月目に再発を来し，以後TAEを7回施行された。前区域に再発した病変が中結腸動脈から供血されTAE無効となった（図3a）。

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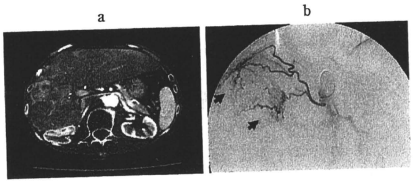

図 1 症例 1
a: 腹部 CT。b: 血管造影。
下横隔動脈からの供血路を認める（矢印）。

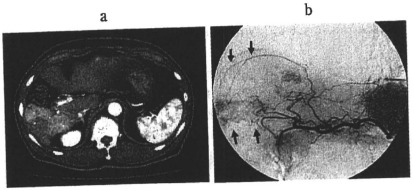

図 2 症例 2
a: 腹部 CT。b: 血管造影。
下横隔動脈，大網からの供血路を認める（矢印）。

症例 4: 60 歳，男性。S7 部分切除後 7 か月目に再発を来し，以後 TAE を 2 回，RFA を 2 回施行されたが，下横隔動脈から供血される TAE 無効病巣が出現した（図 3b）。

これら 4 例の背景を表 1 に，再肝切除術式および術後経過を表 2 に示す。再肝切除前の肝予備能は全例 Child A であった。症例 2，3，4 は横隔膜合併切除を要したが，術中出血量は 1,000 mL 以下であり無輸血で切除可能で

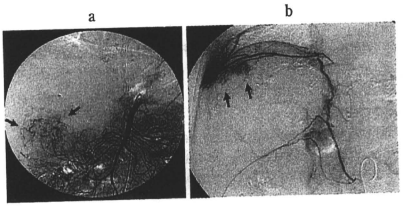

図 3
a: 症例 3，血管造影。
中結腸動脈からの供血路を認める（矢印）。
b: 症例 4，血管造影。
下横隔動脈からの供血路を認める（矢印）。

表 1 臨床的背景

	症例 1	症例 2	症例 3	症例 4
年齢/性別	78 歳/女性	75 歳/男性	70 歳/男性	60 歳/男性
肝予備能	Child A(6 点)	Child A(5 点)	Child A(6 点)	Child A(5 点)
背景肝	C 型慢性肝炎	C 型慢性肝炎	非 B 非 C 肝障害	B 型肝硬変
AFP(ng/mL)	92,180	32	83	3
PIVKA-II(mAU/mL)	75,000	3,542	744	32
初回切除術式	S8 部分切除	S5/6 部分切除	後区域切除	S7 部分切除
初回切除からの期間	10 年 3 か月	5 年 1 か月	3 年 8 か月	1 年 10 か月
再発に対する治療	TAE 9 回 TAI 3 回	TAE 6 回	TAE 7 回	TAE 2 回 RFA 2 回

表 2 再肝切除術式および術後経過

	症例 1	症例 2	症例 3	症例 4
術　式	右葉切除	S6 切除*	前区域切除*	S7 部分切除*
手術時間	4 時間 56 分	5 時間 41 分	6 時間 34 分	6 時間 04 分
出血量	4,410 mL	870 mL	450 mL	500 mL
輸血	あり	なし	なし	なし
術後合併症	なし	SSI	胆汁漏	なし
術後在院日数	12 日	13 日	43 日	12 日
再肝切除後の経過				
後治療	TAE(1 回)	TAI(3 回)	TAE(2 回) 再々肝切除	TAI(1 回) Sorafenib
生存期間	22 か月	20 か月	23 か月	10 か月
転帰	他病死	原病死	原病死	生存

*: 横隔膜合併切除を伴う

あった。症例3は胆汁漏の合併により入院期間が延長したが，他の3例はいずれも術後2週間以内に退院可能であり，重篤な合併症は認めなかった。

観察期間の短い症例4を除く3例は，術後1年以上の生存が得られており，原病死した症例2，3も疼痛などの自覚症状は認めず，QOLは保たれていた。

II. 考　察

多発病変を有する肝細胞癌症例に対する減量肝切除の意義に関しては，いまだ結論は得られておらず，報告例が散見される程度である[1-4]。また，われわれが検索し得た限りでは，「減量再肝切除」に関する報告はなかった。

減量肝切除の意義に関する報告をみると，肝予備能が保たれている症例や門脈内腫瘍栓を伴わない症例では，比較的良好な予後が得られることが示されている。Inoueらは13例の門脈内腫瘍栓を伴わないChild A症例の検討で，1年生存率は67.7%であったと報告している[2]。また，山中らは54例の減量肝切除症例の検討で，3年生存率が50%と報告しているが，門脈内腫瘍栓を伴う症例では予後不良であると述べている[4]。

自験例についてみると，4例はいずれもChild Aで肝予備能は保たれており，また再肝切除前の画像検査で門脈内腫瘍栓を認めた症例はなかった。観察期間の短い症例が含まれているが，現時点での1年生存率は100%であり，短期成績は良好であった。

また今回対象とした4例では重篤な合併症は認めておらず，減量再肝切除は安全に施行可能であった。

以上のことから，肝予備能が良好で門脈内腫瘍栓を認めない症例では，減量再肝切除はTAE無効病巣に対する局所治療の選択肢となり得ると考えられる。

本論文の要旨は第32回日本癌局所療法研究会において発表した。

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サルベージ手術を施行した放射線化学療法後の再発肛門扁平上皮癌の1例

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A Case of Salvage Treatment for Local Recurrence of Squamous Cell Anal Carcinoma after Chemoradiation: Hironobu Goto[*1], Masakazu Ikenaga[*1], Masayoshi Yasui[*1], Michihiko Miyazaki[*1], Hideyuki Mishima[*1], Masanori Tsujie[*1], Atsushi Miyamoto[*1], Motohiro Hirao[*1], Kazumasa Fujitani[*1], Shoji Nakamori[*1], Ken Yoshida[*2] and Toshimasa Tsujinaka[*1] (*1*Dept. of Surgery, and* *2*Dept. of Radiology, National Hospital Organization, Osaka National Hospital*)

Summary

A 76-year-old woman consulted her local physician because she experienced anal pain during defecation. She was diagnosed with squamous cell anal carcinoma and underwent chemoradiation (59.4 Gy+UFT 500 mg/5 days/week). The examinations after chemoradiation revealed a complete remission of the tumor. She was followed up and 8 months later, she experienced anal erosion and pain. Local recurrence was observed, however, distant metastasis was not observed. Abdominoperineal resection (APR) was performed as salvage treatment, and she has been disease free for 10 months. Functional preservation employing concomitant chemoradiation has become the standard treatment for most case of squamous cell anal carcinoma, with APR backup being a salvage procedure. However, approximately 30% of the cases require a salvage operation because of primary non-response or recurrence. We predict that the incidence of such cases will increase in the future. **Key words:** Squamous cell anal carcinoma, Chemoradiation, Salvage treatment

要旨 症例は76歳，女性。肛門部の違和感と排便時の疼痛を自覚し，近医を受診した。生検で肛門扁平上皮癌と診断され，放射線化学療法（UFT 500 mg/5 days/week，59.4 Gy）を行い完全寛解を得た。サーベイランス中の術後8か月目に肛門部の痛みとびらんが出現した。再発を疑い生検を施行したところ，局所再発と診断した。遠隔転移は認めなかった。肛門扁平上皮癌の放射線化学療法後の再発に対してサルベージ手術として，直腸切断術を施行した。術後10か月間経過し，再発を認めず。肛門機能温存が可能な放射線化学療法は，肛門扁平上皮癌に対して標準治療になった。しかし，約30%の症例が放射線化学療法無効，局所再発などによってサルベージ手術を要している。今後は肛門扁平上皮癌に対して放射線化学療法が行われ，必要に応じてサルベージ手術を行う症例が増加すると考えられる。

はじめに

本邦では，肛門扁平上皮癌に対しては直腸切断術が多く選択されてきた。昨今，放射線化学療法による高い有効率，肛門温存率が報告され[1-3]，初回治療は放射線化学療法が標準治療となっている。今回われわれは，肛門扁平上皮癌に放射線化学療法を施行し，完全寛解(complete remission: CR)後に局所再発を来し，サルベージ手術を行った1例を経験したので報告する。

I. 症　例

患者: 76歳，女性。

主訴: 肛門部の痛み，びらん。

既往歴: 特記すべきことなし。

現病歴: 2007年8月ごろから肛門部の違和感を自覚し，2008年8月ごろから排便時の疼痛を自覚し，近医を受診した。

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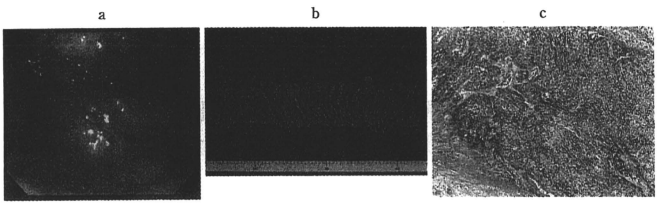

図 1
a: 肛門管直上に 2/3 周性の隆起性病変を認め，腫瘍の肛門側は歯状線に及んでいた。
b: 肛門管に隆起性病変を認めた。
c: squamous cell carcinoma の像を呈し，筋層まで浸潤を認めた（HE 染色×40・原倍率）。

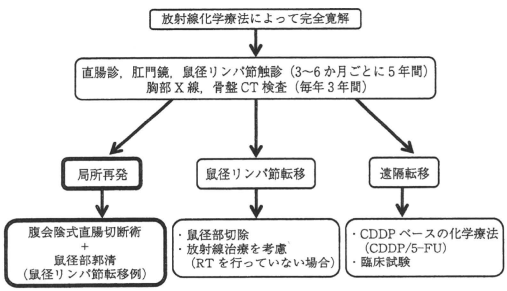

図 2 肛門管扁平上皮癌に対する NCCN（National Comprehensive Cancer Network）のフォローアップ/サーベイランス指針（2010 年）

下部消化管内視鏡検査: 肛門管直上に 2/3 周性の隆起性病変を認め，腫瘍の肛門側は歯状線に及んでいた（図 1a）。生検で肛門扁平上皮癌と診断された。

その他の画像検査でリンパ節の腫大，遠隔転移を認めなかったため，肛門扁平上皮癌（P，5 型，30×30 mm，cMPcN0cM0，cStage II）と診断した。

放射線化学療法（UFT 500 mg/5 days/week，59.4 Gy）を施行し，効果判定は CR と判定した。サーベイランス中の 2009 年 6 月ごろから肛門部の痛みとびらんが出現し，直腸診でも隆起性病変を触知した。

血液検査: 血清 SCC 値は 1.1 ng/mL と上昇を認めなかった。血算，生化学検査ともに特記すべき異常を認めなかった。

腹部造影 CT 検査: 遠隔転移，リンパ節再発を疑わす所見を認めなかった。

FDG-PET 検査: 肛門のみに FDG の集積（SUVmax 4.0）を認めた。

以上より，肛門扁平上皮癌に対する放射線化学療法後の局所再発を疑い，腰椎麻酔下に生検を行ったところ肛門扁平上皮癌と診断した。サルベージ手術として腹会陰式直腸切断術（abdominoperineal resection: APR）を施行した。

切除標本肉眼所見: 肛門管に隆起性病変を認めた（図 1b）。

病理組織学的所見: squamous cell carcinoma の像を呈し，筋層まで浸潤を認めた（図 1c）。

術後経過: 会陰部の創治癒遅延を認めたが，保存的治療で改善した。現在，10 か月間再発を認めず，経過観察中である。

II. 考　察

これまで，肛門扁平上癌に対しては APR が行われ，5 年生存率は 33〜90％であった[1,4]。1980 年代から放射線化学療法により 5 年生存率 56〜92％の治療成績が報告された[1-3]。高い肛門温存率により QOL の改善を伴うことから，標準治療として受け入れられるようになった。しかし上記の治療成績は，放射線化学療法無効例，放射線化学療法後の再発例に対するサルベージ手術による効果

を含む成績である。約30%の症例が放射線化学療法無効，局所再発などによってサルベージ手術を要している[5]（図2）。サルベージ手術後の5年生存率44～60%と報告されている[1]。本症例では放射線化学療法を行い，CRを得た後，8か月後に局所再発を認めたが，適切にサーベイランスを施行することでサルベージ手術を行うことができた。

医学中央雑誌にて，「肛門癌」，「放射線化学療法後再発」をキーワードに検索したところ，肛門扁平上皮癌の放射線化学療法後の再発に対してサルベージ手術を行った本邦報告例は1例のみであった[5]。今後は肛門扁平上皮癌の初回治療には放射線化学療法が選択され，必要に応じてサルベージ手術を行う症例が増加すると考えられる。

肛門扁平上皮癌に対する放射線化学療法は，良好な治療成績と高い肛門温存率を得ることができるが，その成績は厳密な経過観察と約30%の症例で生じる局所再発に対するサルベージ手術によって得られていることを忘れてはならない。

結　語

われわれは肛門扁平上皮癌に対して，放射線化学療法を行いCR後に局所再発を来し，サルベージ手術を行った1例を経験したので報告した。

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Approach to Oncology

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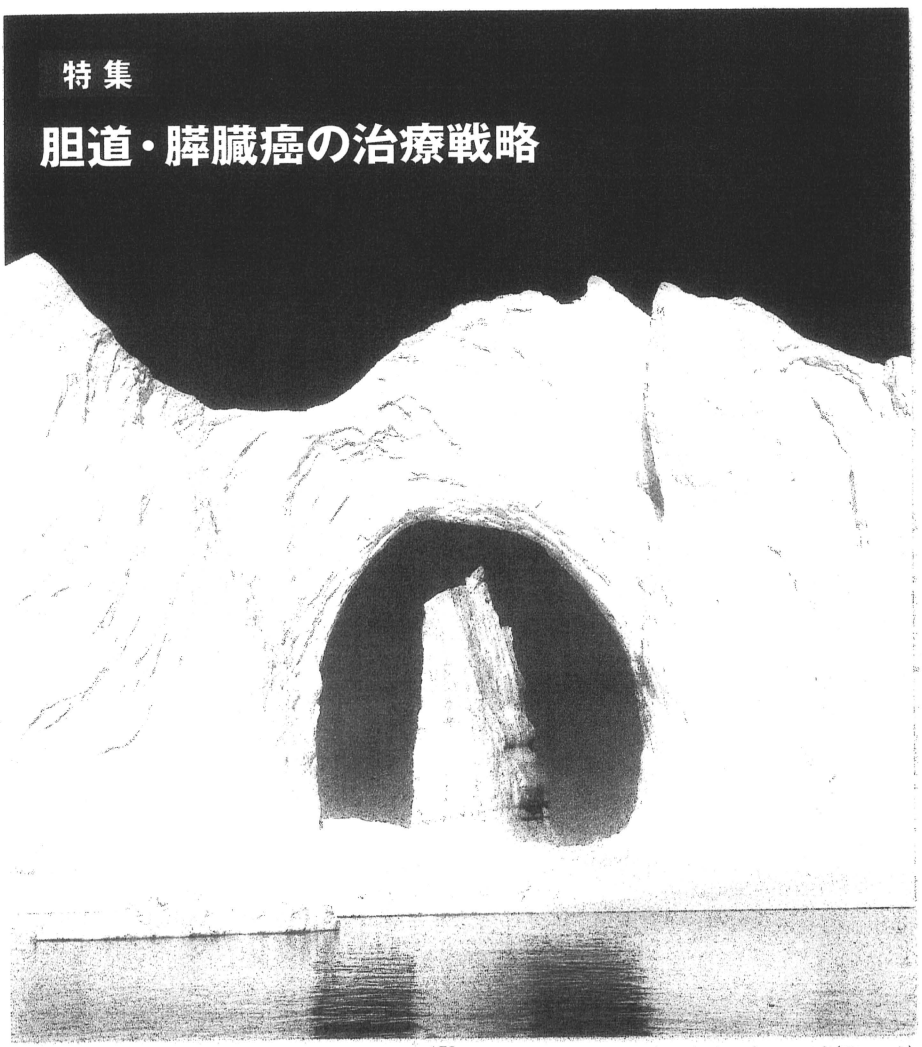
特集

胆道・膵臓癌の治療戦略



イルリサット・アイスフィヨルド（デンマーク）

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