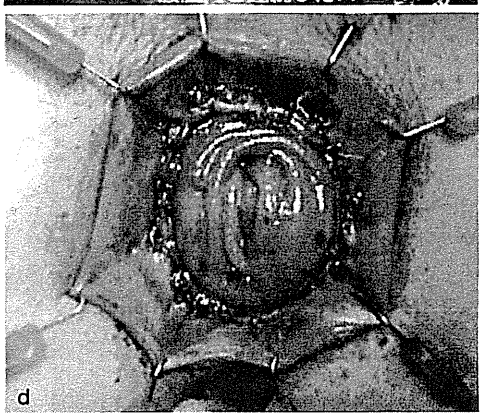

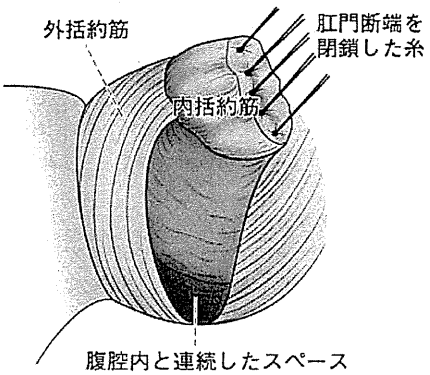

図6 (2)　肛門からのintersphincteric dissection

c) 腹腔内との連結。
腹腔内から十分剝離されていれば，比較的すみやかに肛門側と腹腔内側のintersphincteric planeはつながる。通常後壁で連続させることが多い。一度腹腔内とつながるとその部位を手掛かりにスペースを広げて後壁から側壁へと腹腔内とのスペースを広げる。
d) 結腸肛門吻合。
最初の8針はマットレス縫合で粘膜を十分合わせる。つぎにその間を2針ずつ全層にかけると全体で24針縫合することになる。

まずは，切離予定粘膜に電気メスでマーキングする。後壁で外肛門括約筋の輪状線維が見えるまで直腸粘膜と内肛門括約筋を切る。一度外括約筋が露出したら，今度は超音波凝固切開装置のtissue padを内外括約筋間に滑り込ませ，全周にわたり，内外括約筋間を剝離する（図6a）。以前は電気メスでこの手技を行っていたが，少なからず内痔核を有する症例も多くここでの手術操作で出血を最小限にとどめるためにこの方法は有用である。その後直腸断端を縫合し，癌細胞の散布を防ぐようにする。つぎにE式開肛器をかけて，肛門を広く展開し，後壁近傍よりintersphincteric planeを剝離する（図6b）。最近では多くの場合腹腔内から十分剝離が終了しているため，後壁の1カ所でPéanなどで内外括約筋間を剝離するとすぐに腹腔側と連続される。どこか1カ所でつながったら，その空間を手掛かりとして後壁から左右側壁を広く腹腔内と連結させる（図6c）。この手技は腹腔内から十分剝離されていればそうむずかしい手技ではない。最後に前壁が残ることになる。直腸前壁は，intersphincteric planeはわかりにくく，前立腺あるいは膣後壁を目安として剝離を行う。つまり男性では前立腺のツルッとした被膜が露出されたらこれに沿って剝離する。前立腺側に剝離層がずれた場合には出血するので，誤った剝離層を是正すべきである。女性の場合には膣後壁と外括約筋の境界を区別することはきわめてむずかしく，時に膣に指を入れて膣後壁の厚さを感じながら前壁での剝離操作をすることもある。

これらによる全周で腹腔内と連結されたら，標本を肛門側より引出し，肛門側より口側腸管をリニアカッターで切離する。吻合予定腸管の左右断端に糸をかけ，いったん腹腔内に戻す。その後腹腔側のポートより洗浄デバイスを入れ，先端を肛門管近傍におく。腹腔側より水を出し，肛門管を十分洗い流して，肛門側より水を回収する。これにより吻合近傍の遊離癌細胞を除く効果を期待し

ている。そのあと，postanal repairを行ったのち，先ほどかけた糸を引き，口側腸管を肛門へと誘導する。

その後結腸肛門吻合（coloanal anastomosis：CAA）を行う。初めは45度ずつ8針マットレス縫合を行い，粘膜同士をきれいに合わせる。つぎに間を2針ずつ入れていくと計24針の手縫い吻合が完成する（図6d）。Ileostomyを造設しない場合には，経肛門的にドレーンを挿入することが多い。その後腹部操作に戻り，左上のポート部より19Fr JVAC suctionドレーンを骨盤底に留置し，手術を終える。閉創は通常縫合で行う。

おわりに

腹腔鏡下ISRの手術手技を習熟することは，腫瘍学的安全性を担保すると同時に，余計な括約筋損傷や切除を回避することに直結する。すなわち，癌を治しながら，合併症なく，かつ機能損傷を最小限とするために，内視鏡外科技術を日々磨く必要がある分野である。十分な外科解剖の理解や各手技局面での定型化された手術方法を確立して，より低侵襲な肛門温存手術が広く提供されることを期待する。

Suppression of heat shock protein 27 expression promotes 5-fluorouracil sensitivity in colon cancer cells in a xenograft model

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Abstract. The present study investigates whether the expression levels of heat shock protein 27 (HSP27) in colon cancer cells are associated with 5-fluorouracil (5-FU) sensitivity in a xenograft model, as well as the mechanism responsible for regulating 5-FU sensitivity. HCT116 cells which have a high expression of HSP27 were stably transfected with specific short hairpin RNA (shRNA) in order to suppress HSP27 expression. The association between HSP27 protein expression levels and 5-FU sensitivity was evaluated in a mouse xenograft model. The mRNA expression of 5-FU metabolic enzymes and cell apoptosis were also analyzed in the transfected cells. The suppression of HSP27 protein expression led to enhanced 5-FU sensitivity. The mRNA expression levels of dihydropyrimidine dehydrogenase and orotate phosphoribosyltransferase, but not those of thymidylate synthase, and the number of apoptotic cells increased in the transfected cells after 5-FU exposure. In conclusion, the suppression of HSP27 expression in colon cancer cells may promote 5-FU sensitivity by inducing apoptosis, despite the acceleration in 5-FU metabolism.

Introduction

Over the last few decades, systemic chemotherapy for colon cancer has markedly changed worldwide. Combinations of 5-fluorouracil (5-FU) with other cytotoxic agents, such as oxaliplatin and irinotecan, have improved the prognosis of patients with advanced colon cancer. Furthermore, the addition of molecular-targeted agents, such as anti-VEGF antibody (bevacizumab) and anti-EGFR antibody (cetuximab/panitumumab), have resulted in dramatic improvements in the survival of patients with advanced colon cancer and are currently regarded as first-line chemotherapy regimens (1-4). Although 5-FU is still a key drug in these regimens, the inherent or acquired resistance to 5-FU in colon cancer is a critical problem. A number of studies have reported that 5-FU metabolism-related factors, such as thymidylate synthase (TS), folate co-factors, dihydropyrimidine dehydrogenase (DPD) and orotate phosphoribosyltransferase (OPRT), are associated with the response to or toxicity of 5-FU (5-8). However, there are still no reliable biomarkers for the sensitivity or resistance to 5-FU chemotherapy.

Mammalian heat shock proteins (HSPs) have been classified into four main families based on their molecular weights: HSP90, HSP70, HSP60, and small HSPs (15-30 kDa), including HSP27. These proteins are well known as molecular chaperons in protein-protein interactions, as anti-apoptotic proteins and contributors to cell survival (9,10). Many studies have reported that HSP27 expression contributes to the malignant properties of cancer cells, including tumorigenicity, treatment resistance, and apoptosis inhibition (11-15). In colon cancer, certain studies have reported that HSP27 expression participates in the resistance to doxorubicin or irinotecan *in vitro* (16,17), and HSP27 has recently been considered as a prognostic marker in clinicopathological studies (18-20). Our previous study also indicated that HSP27 expression participates in the degree of resistance to 5-FU, a key drug for the treatment of colorectal cancer, in experiments performed *in vitro* (21). In the present study, we sought to clarify whether HSP27 can be used as a clinical biomarker for 5-FU chemotherapy or as a treatment target for 5-FU resistance using a xenograft model.

Materials and methods

Drug, cell lines and cell culture conditions. The anticancer drug, 5-FU, was purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). The human colon cancer cell line, HCT116, was obtained from the American Type Culture Collection (Rockville, MD). The cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, MA). Each culture was supplemented with 10% fetal bovine serum (CSL Ltd., Melbourne, Australia) and 1% penicillin/streptomycin. The cells were cultured at 37°C with 5% CO_2.

Stable transfection with short hairpin RNA (shRNA). An oligonucleotide for a short hairpin RNA targeting the human

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HSP27 site (5'-UAGCCAUGCUCGUCCUGCCUU-3') was designed (21), so as to contain 5'-*Bam*HI and 3'-*Eco*RI overhangs. The annealed oligonucleotide was then ligated into a linearized U6 promoter-driven vector (pSIREN-DNR-DsRed-Express Vector; Clontech, Mountain View, CA). As the negative control, an oligonucleotide for a scrambled shHSP27 (5'-ACGUAAGGCGCGUAACGGGTT-3') containing 5'-*Bam*HI and 3'-*Eco*RI overhangs was also constructed and ligated to the pSIREN vector.

For transfection, HCT116 cells expressing high levels of HSP27 under normal culture conditions were plated in 6-well plates at a density of $1x10^6$ cells per well and allowed to grow overnight. The cells were transfected with a mixture of 5 μg of siRNA-encoding plasmids (the HSP27 target sequence plasmid and the negative control plasmid) and 20 μl of Lipofectamine 2000 (Invitrogen Life Technologies, Inc., Carlsbad, CA) in 2 ml of serum-free medium, according to the manufacturer's instructions. After incubation at 37°C for 6 h, the medium was replaced with fresh growing medium. The transfected cells, containing a red fluorescent protein, were verified by visualization in living cells using fluorescence microscopy. Some of the transfected clones with different levels of HSP27 expression were monoclonally obtained and named HCT116-shRNA/HSP27-1 to -4. The control cells, HCT116-scramble/HSP27 and HCT116-mock cells, were established in a similar manner.

Quantitative real-time PCR (qRT-PCR) analysis of 5-FU metabolic enzymes. Total RNA was extracted from the cells in each group, and qRT-PCR analysis was performed using a fluorescence-based, real-time detection method [ABI PRISM 7900 Sequence Detection System (TaqMan); Applied Biosystems, Foster City, CA]. The mRNA expression of DPD, OPRT and TS was analyzed in the transfected cells that were either treated or not with 5-FU (1.28 μg/ml) in culture medium for 24 h. The enzyme expression levels of the transfected cells were assessed relative to the levels of the gene transcript in the mock transfected cells (interest/β-actin). The sequences of the primers and probes were as follows: DPD: 51F (19 bp), AGGACGCAAGG AGGGTTTG; 134R (20 bp), GTCCGCCGAGTCCTTACTGA; probe-71T (29 bp), CAGTGCCTACAGTCTCGAGTCTGCC AGTG. OPRT: 496F (25 bp), TAGTGTTTTGGAAACTG TTGAGGTT; 586R (20 bp), CTTGCCTCCCTGCTCTCTGT; probe-528T (27 bp), TGGCATCAGTGACCTTCAAGCCC TCCT. TS: 764F (18 bp), GCCTCGGTGTGCCTTTCA; 830R (17 bp), CCCGTGATGTGCGCAAT; probe-785T (21 bp), TCGCCAGCTAC GCCCTGCTCA. β-actin: 592F (18 bp), TGAGCGCGGCTACAGCTT; 651R (22 bp), TCCTTAA TGTCACGCACGATTT; probe-611T (18 bp), ACCACCA CGGCCGAGCGG. The 25-μl PCR reaction mixture contained 600 nmol/l of each primer, 200 nmol/l each of dATP, dCTP and dGTP, 400 μmol/l of dUTP, 5.5 μmol/l of $MgCl_2$ and 1X TaqMan buffer A containing a reference dye (all reagents were supplied by Applied Biosystems). The PCR conditions were 50°C for 10 sec and 95°C for 10 min, followed by 42 cycles at 95°C for 15 sec and 60°C for 1 min.

Western blot analysis. Total cell lysates were extracted with lysis buffer with standard methods. The quantity of cell lysates was determined using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA), and 20 mg of lysates in total were resolved in Ready Gel (Bio-Rad Laboratories) and transferred to an Immuno-Blot™ polyvinylidene fluoride membrane (Bio-Rad Laboratories). The membrane was blocked in phosphate-buffered saline (PBS) containing 5% non-fat milk powder for 2 h at room temperature, then incubated at 4°C overnight with 1:2000-diluted anti-human HSP27 mouse monoclonal antibody (G3.1; Lab Vision Corp., Fremont, CA) or 1:5000-diluted anti-human β-actin mouse monoclonal antibody (AC74; Sigma, St. Louis, MO). The membranes were incubated for 30 min with a 1:5000-diluted horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG) (Promega Corp., Madison, WI). Bound complexes were detected using the ECL-Plus reagent (Amersham Biosciences Corp., Cardiff, UK) according to the manufacturer's instructions. The density of the band was measured using NIH imaging (Scion) software and normalized to β-actin. Each experiment was performed in triplicate.

Xenograft model. Female nude mice with a BALB/cA genetic background were purchased from CLEA Japan Co. Ltd., Tokyo, Japan. The mice were maintained under specific pathogen-free conditions using an Isorack in our experimental animal center and fed sterile food and water. Six- to eight-week-old mice weighing 20-22 g were used for the experiments. To analyze the effect of HSP27 knockdown against 5-FU treatment, HCT116-mock, HCT116-scramble/HSP27, and HCT116-shRNA/HSP27 cells ($3x10^6$ cells per injection), which were resuspended in 100 μl of PBS, were injected into the subcutaneous tissues of the bilateral dorsum of ether-anesthetized mice using a 1-ml syringe and a 27-gauge tuberculin needle (Terumo Co., Tokyo, Japan). The tumors were measured (length and width) twice a week using a sliding caliper by the same observer. When the inoculated tumors reached 5 mm in length, 5-FU (200 μl saline solution) was administered intraperitoneally at a dose of 60 mg/kg on days 1, 5 and 9 (n=5 mice per group). As the control, saline (200 μl) was also administered intraperitoneally (n=5 mice). The estimated tumor volume (EV) was calculated as EV = length x width^2 x 1/2. EV was plotted against the day since 5-FU treatment initiation to derive a xenograft growth curve. All the mice were sacrificed at three weeks after the initial treatment. Tumors were collected and fixed in 4% paraformaldehyde (Sigma) at room temperature for 24 h before processing for sectioning and immunohistochemical staining or the preparation of tumor lysates for western blot analysis. All animal studies were conducted in accordance with the institutional guidelines approved by the Animal Care and Use Committee of our university.

Immunohistochemical staining. Anti-human HSP27 rabbit polyclonal antibody (Stressgen Bioreagents Corp., Victoria, BC, Canada) was used for the immunohistochemical staining of the tumors from the mice. Immunohistochemical staining was performed according to the standard streptavidin-biotin peroxidase complex method using a Histofine™ SAB-PO[M] kit (Nichirei, Tokyo, Japan). Briefly, deparaffinized sections were placed in methanol containing 1% hydrogen peroxide for 15 min to block endogenous peroxidase activity. After washing with PBS, the sections were incubated with anti-HSP27 antibody (1:100 diluted in PBS) overnight at 4°C in a moist chamber, and then

incubated with biotinylated goat anti-rabbit IgG and peroxidase-conjugated streptavidin (Stressgen Bioreagents Corp.) for 30 min each at room temperature. After a final washing with PBS, the sections were immersed in a PBS solution containing 0.02% 3,3'-diaminobenzidine and 0.1% hematoxylin and mounted in balsam.

Detection of caspase-3 activities for apoptosis analysis. The transfected cells were treated with or without 5-FU (1.28 μg/ml) in the culture medium for 24 h. The caspase-3 activities were then measured using an ApoAlert Caspase-3 Colorimetric assay kit (Clontech), according to the manufacturer's instructions. The cells ($2x10^6$) were centrifuged at 400 x g for 5 min. After the extraction of the cell lysates using cell lysis buffer, the supernatants were centrifuged for 10 min at 4°C to precipitate the cellular debris and were then incubated with reaction buffer/dithiothreitol (DTT) mix for 30 min on ice and caspase-3 substrate for 1 h at 37°C. The samples were read at 405 nm on a microplate reader.

Statistical analysis. Each value was expressed as the mean ± standard deviation. Statistical analysis was performed using the Student's t-test or the Mann-Whitney U test. The regression analysis was performed using the Statistical Package for Social Sciences (SPSS) 13.0J for Windows (SPSS, Chicago, IL). $P<0.05$ was considered to indicate a statistically significant difference.

Results

5-FU sensitivity of shRNA/HSP27-transfected cells in vitro. The 5-FU-resistant colon cancer cells, HCT116, were transfected with HSP27 shRNA ligated to the pSIREN-DNR-DsRed-Express vector (shRNA/HSP27-transfected cells), scrambled shRNA ligated to the pSIREN vector as the control (scramble/HSP27-transfected cells), or an empty vector (mock-transfected cells). Transfected cells with various HSP27 expression levels were obtained by cloning (Fig. 1; shRNA/HSP27-1 to -4). To evaluate the 5-FU sensitivity, the transfected cells were first treated with 5-FU *in vitro*, and then the IC_{50} of each transfectant was measured using an MTT assay. A significant correlation between the HSP27 protein levels and the IC_{50} of 5-FU was observed (Fig. 2; R=0.931, P=0.0039).

5-FU sensitivity of shRNA/HSP27-transfected cells in xenograft model. To evaluate the 5-FU sensitivity in the xenograft model, the transfected cells were injected into the subcutaneous tissues of the bilateral dorsum of ether-anesthetized mice. When the inoculated tumors reached 5 mm in length, 5-FU was administered intraperitoneally at a dose of 60 mg/kg on days 1, 5 and 9 (n=5 mice per group). Saline (200 μl) was also administered intraperitoneally in the control group (n=5 mice). The EV following 5-FU treatment was significantly suppressed in the mice injected with the shRNA/HSP27-transfected cells, compared to those injected with the scramble/HSP27- and mock-transfected cells (Figs. 3 and 4).

Immunohistochemistry of the tumors in the xenograft model showed high expression levels of HSP27 in the mock- and scramble/HSP27-transfected cells, but not in the shRNA/HSP27-transfected cells (Fig. 5).

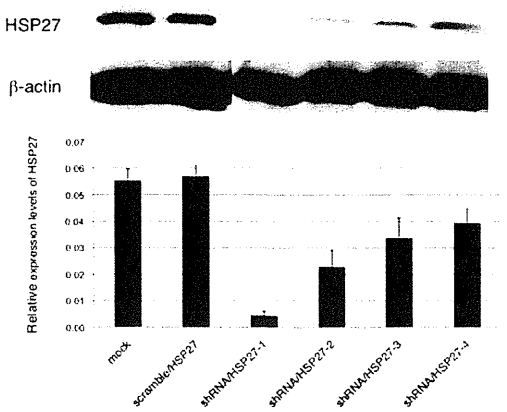

Figure 1. HSP27 protein expression in the shRNA-transfected cells. The 5-FU-resistant colon cancer cells, HCT116, were transfected with shRNA of HSP27. Western blot analysis showed different suppression levels of HSP27 in the transfected cells (shRNA/HSP27-1 to -4). Relative expression levels of HSP27 were assessed by measuring the densities of the bands using NIH image software, and normalized to β-actin.

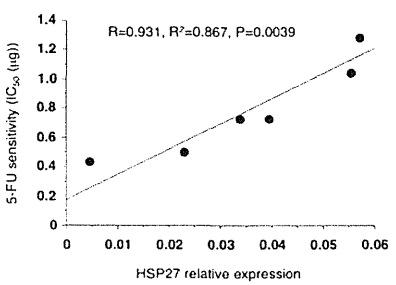

Figure 2. Correlation between HSP27 expression and 5-FU sensitivity (IC_{50}) in the shRNA-transfected cells. A significant correlation between HSP27 protein expression and 5-FU sensitivity (IC_{50}) by MTT assay was observed in the transfected cells.

Expression of 5-FU metabolic enzymes in shRNA/HSP27-transfected cells. To evaluate whether HSP27 expression levels affect the principal 5-FU metabolic enzymes (TS, DPD and OPRT) in the transfected cells treated with 5-FU, a qRT-PCR analysis of 5-FU metabolic enzymes was performed. These enzyme expression levels were assessed relative to the levels of the gene transcript in the mock-transfected cells as the control. Although no significant differences in the mRNA expressions of the three metabolic enzymes were observed between the shRNA/HSP27-transfected cells and the mock- or scramble/HSP27-transfected cells, the expression of DPD and OPRT was higher in the shRNA/HSP27-transfected cells (Fig. 6). Under the same conditions with 5-FU treatment, apoptosis induction was more frequently observed in the shRNA/HSP27-transfected cells than in the mock- or scramble/HSP27-transfected cells (Fig. 7).

Discussion

The inherent or acquired resistance to 5-FU-based chemotherapy remains a critical issue in colorectal cancer treatment. Consequently, the identification of biomarkers for chemosensitivity or resistance, such as the *K-RAS* and *B-RAF* mutation

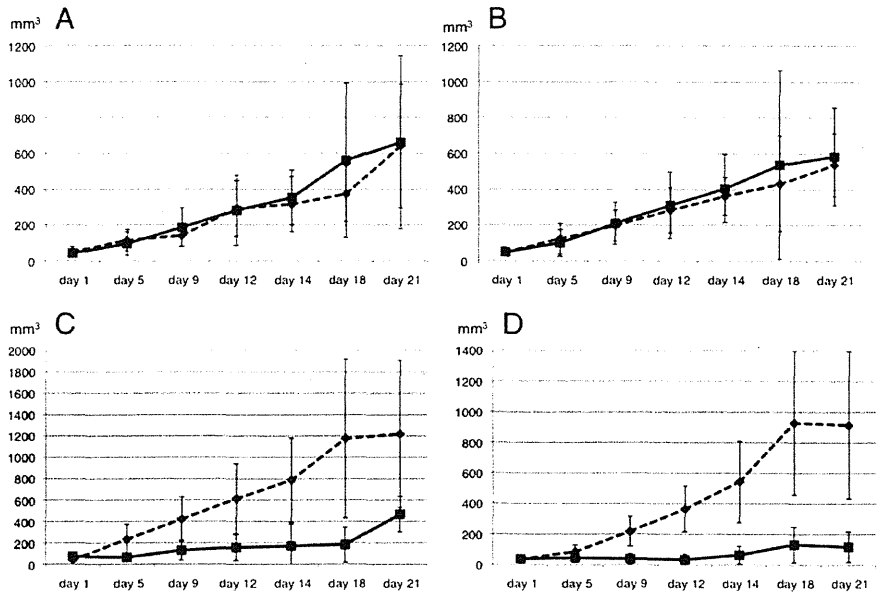

Figure 3. 5-FU sensitivity of the HSP27/shRNA-transfected cells in a xenograft model. Cells ($3x10^6$) were inoculated in the dorsal subcutaneous tissues of nude mice. When the inoculated tumors reached 5 mm in length, 5-FU (60 mg/kg/200 ml saline solution) was administered intraperitoneally on days 1, 5 and 9 (black line). Estimated tumor volumes were calculated until 21 days from initial administration of 5-FU. As the control, saline (200 ml) was also administered intraperitoneally (dotted line). (A) Mock-transfected cells. (B) Scramble/HSP27-transfected cells. (C) shRNA/HSP27-1-transfected cells. (D) shRNA/HSP27-2-transfected cells.

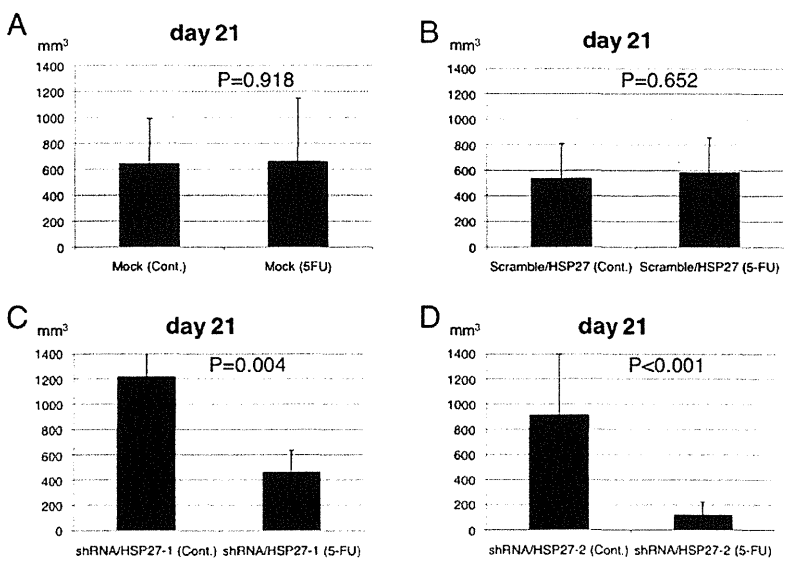

Figure 4. 5-FU sensitivity of the HSP27/shRNA-transfected cells on day 21 in the xenograft model. Estimated tumor volumes were calculated on day 21 after the intraperitoneal injection of 5-FU. 5-FU (60 mg/kg/200 ml saline solution) was administered intraperitoneally on days 1, 5 and 9. As the conrol, saline (200 ml) was also administered intraperitoneally. (A) Mock-transfected cells. (B) Scramble/HSP27-transfected cells. (C) shRNA/HSP27-1-transfected cells. (D) shRNA/HSP27-2-transfected cells.

status for anti-EGFR antibody, is important for personalized chemotherapy and the avoidance of unsuitable chemotherapy and adverse events. In addition, the identification of a new treatment strategy for patients with resistance to chemotherapy is required to improve the survival of colorectal cancer patients.

HSP27 has been widely recognized as a stress-activated, ATP-independent cytoprotective chaperone that is associated with a number of functions, including chemoresistance in several cancers. In colorectal cancer, HSP27 has been reported as a clinical prognostic factor or as a factor of irinotecan resistance in experiments performed *in vitro* (17-20). Our previous study also reported that the overexpression of HSP27 reduced 5-FU sensitivity and the suppression of HSP27 expression reduced 5-FU resistance in experiments performed *in vitro* using colon cancer cells (21). Furthermore, in prostate and bladder cancer cells, the inhibition of HSP27 expression reportedly enhanced drug sensitivity in xenograft models (22-24). Thus, HSP27 is considered to be a predictor of cancer prog-

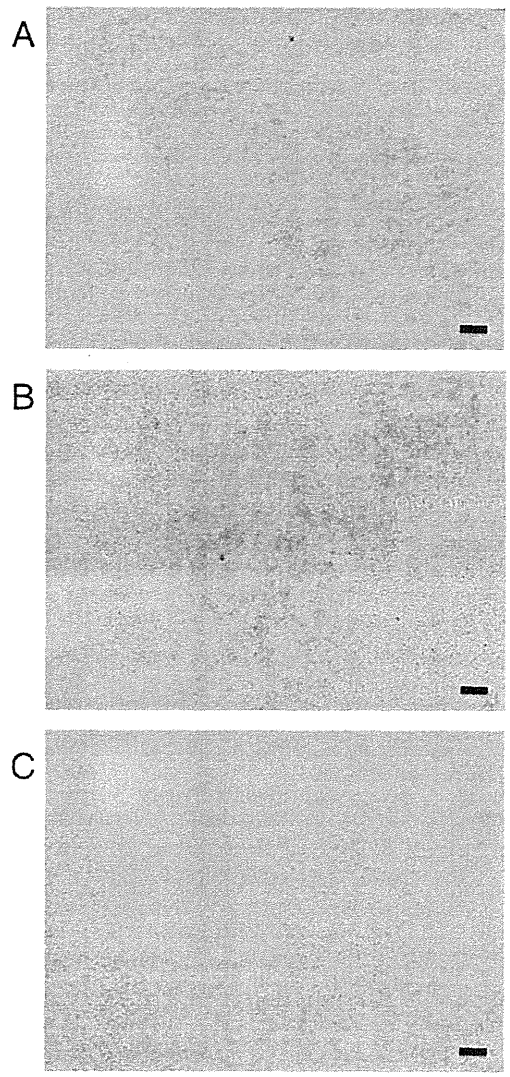

Figure 5. Histology images of the inoculated tumors. Immunohistochemical staining with the anti-HSP27 antibody revealed high expression levels in the mock- and scramble/HSP27-transfected cells, but not in the shRNA/HSP27-1-transfected cells. (A) Mock-transfected cells. (B) Scramble/HSP27-transfected cells. (C) shRNA/HSP27-1- transfected cells. Bar, 50 mm.

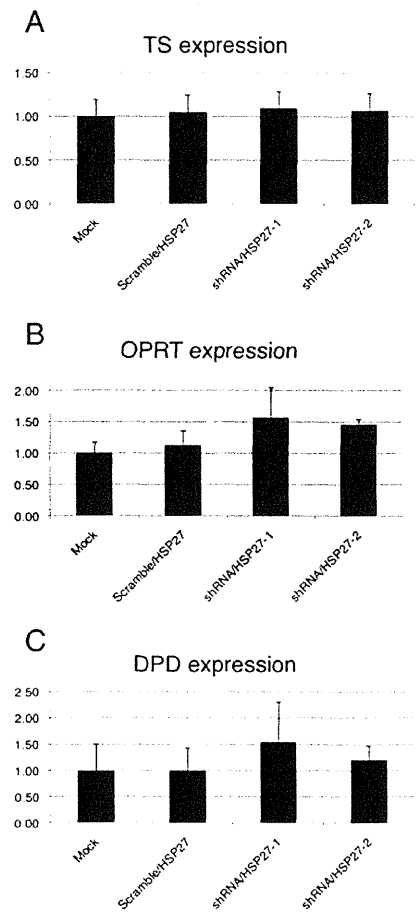

Figure 6. mRNA expression levels of principal 5-FU metabolic enzymes in the shRNA/HSP27-transfected cells. mRNA expression levels of the three enzymes were assessed relative to the levels of the gene transcript in the mock-transfected cells as the control. (A) TS mRNA expression. (B) OPRT mRNA expression. (C) DPD mRNA expression.

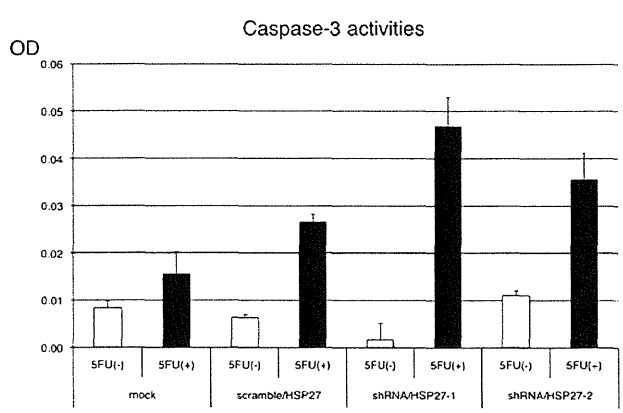

Figure 7. Apoptosis induction by 5-FU treatment in the shRNA/HSP27 transfected cells. Caspase-3 activities were measured on a microplate reader at 405 nm using the ApoAlert Caspase-3 Colorimetric assay kit. White bar, no treatment condition as the negative control; black bar, 5-FU treatment condition; OD, optical density.

nosis or a treatment target for several cancers; however, the role of HSP27 in colorectal cancer, including chemoresistance, remains uncertain due to the lack of evidence in xenograft models. Recently, a phase I/II clinical trial of monotherapy using the antisense oligonucleotide (ASO), OGX-427, which inhibits HSP27 expression, in patients with prostate, bladder, ovarian, breast, or non-small cell lung cancer, but not colorectal cancer, was carried out in the United States and Canada, and the therapy proved to be feasible and effective [J Clin Oncol 28 (Suppl): S15, abs. 3077, 2010]. Accordingly, the purpose of this study was to verify that HSP27 can also serve as a target for the treatment of colorectal cancer.

The present study demonstrates that the suppression of HSP27 expression in HSP27 high-expressing colon cancer cells reduces resistance to 5-FU chemotherapy in a xenograft model, and that the induction of apoptosis caused by the suppression of HSP27 expression, which many studies have reported, may be connected to 5-FU sensitivity. Our findings are consistent with the findings of several studies that link HSP27 and chemoresistance in

different cell types. Collectively, this evidence suggests a role of HSP27 in the mediation of 5-FU resistance in colon cancer cells. HSP27 may also serve as a reliable target for the clinical treatment of colon cancer in patients with chemoresistance.

5-FU is well known to induce apoptosis in colon cancer cells, predominantly through the mitochondrial pathway, involving the release of cytochrome *c* and the subsequent activation of the upstream initiator, caspase-9, and the downstream effector, caspase-3 (25-27). We confirmed that the suppression of HSP27 expression was associated with the induction of apoptosis in colon cancer cells treated with 5-FU. Consequently, HSP27 may function as a negative regulator of the cytochrome *c*-dependent activation of procaspase-3, as previously reported (28). However, whether HSP27 is associated with 5-FU metabolic enzymes, remains unknown. We then analyzed the correlation between HSP27 expression and the principal 5-FU metabolic enzymes, such as TS, DPD and OPRT. The mRNA expression of DPD and OPRT was higher after 5-FU treatment in the shRNA/HSP27-transfected cells that had a high sensitivity to 5-FU comapred to the control cells, although the difference was not significant. Changes in the TS mRNA expression levels were not clearly observed in the shRNA/HSP27-transfected cells. DPD mediates 5-FU degradation by catabolizing 5-FU to fluoro-5,6-dihydrouracil. The enzyme OPRT directly converts 5-FU to 5-fluorouridine-5'- monophosphate and inhibits normal RNA or DNA synthesis in tumor cells. TS is inhibited by 5-fluoro-2'-deoxyuridine-5'- monophosphate (FdUMP) derived from 5-FU, leading to the inhibition of DNA synthesis. Consequently, high TS levels in tumor tissues are considered to be associated with the low efficacy of 5-FU treatment. In consideration of the functions of these enzymes, shRNA/HSP27-transfected colon cancer cells (with a suppressed HSP27 expression) may promote 5-FU metabolism by increasing the expression of DPD and OPRT to remove 5-FU from the cytoplasm, in order to enable cell survival.

In conclusion, this study suggests that the suppression of HSP27 expression in colon cancer cells promotes 5-FU sensitivity by inducing apoptosis, while also accelerating 5-FU metabolism to avoid cell death. Further investigation, such as an HSP27 functional study examining HSP27 phosphorylation activity and signal regulation, may lead to novel treatments for colorectal cancer.

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SPECIAL ARTICLE

Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer

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Abstract Colorectal cancer is a major cause of death in Japan, where it accounts for the largest number of deaths from malignant neoplasms in women and the third largest number in men. Many new treatment methods have been developed over the last few decades. The Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer (JSCCR Guidelines 2010) have been prepared to show standard treatment strategies for colorectal cancer, to eliminate disparities among institutions in terms of treatment, to eliminate unnecessary treatment and insufficient treatment, and to deepen mutual understanding between health-care professionals and patients by making these Guidelines available to the general public. These Guidelines have been prepared by consensuses reached by the JSCCR Guideline Committee, based on a careful review of the evidence retrieved by literature searches and in view of the medical health insurance system and actual clinical practice settings in Japan. Therefore, these Guidelines can be used as a tool for treating colorectal cancer in actual clinical practice settings. More specifically, they can be used as a guide to

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obtaining informed consent from patients and choosing the method of treatment for each patient. As a result of the discussions held by the Guideline Committee, controversial issues were selected as Clinical Questions, and recommendations were made. Each recommendation is accompanied by a classification of the evidence and a classification of recommendation categories based on the consensus reached by the Guideline Committee members. Here we present the English version of the JSCCR Guidelines 2010.

Keywords Colorectal cancer · Guideline · Treatment · Surgery · Chemotherapy · Endoscopy · Radiotherapy · Palliative care · Surveillance

Introduction

1. Guideline objectives

Mortality and morbidity from colorectal cancer have substantially increased in Japan recently. According to the vital statistics for Japan in 2008, colorectal cancer accounted for the largest number of deaths from malignant neoplasms in women and the third largest number in men, after lung cancer and gastric cancer. Nevertheless, the number of deaths from colorectal cancer per unit population has increased approximately tenfold during the past 50 years. Many new treatment methods have been developed during that time, and their use in combination with advances in diagnostic methods has led to a steady improvement in the results of treatment. However, there are differences in treatment among medical institutions in Japan that provide medical care for patients with colorectal cancer, and these differences may lead to differences in the results of treatment.

Under such circumstances, the JSCCR guidelines 2010 for the treatment of colorectal cancer (JSCCR Guidelines 2010), which are intended for doctors (general practitioners and specialists) who provide medical care for patients with colorectal cancer at various disease stages and conditions, have been prepared for the following purposes: (1) to show standard treatment strategies for colorectal cancer; (2) to eliminate disparities among institutions in terms of treatment; (3) to eliminate unnecessary treatment and insufficient treatment; and (4) to deepen mutual understanding between health-care professionals and patients by making these Guidelines available to the general public [1].

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The following are expected to be achieved with these Guidelines: (1) improved treatment of colorectal cancer in Japan; (2) improved results of such treatment; (3) reduced human and financial burdens; and (4) increased benefits for patients.

2. How to use these Guidelines

These Guidelines have been prepared by consensuses reached by the JSCCR Guideline Committee, based on a careful review of the evidence retrieved by literature searches and in view of the medical health insurance system and actual clinical practice settings in Japan, so these Guidelines can be used as a tool for treating colorectal cancer in actual clinical practice settings. More specifically, they can be used as a guide to obtaining informed consent from patients and choosing the method of treatment for each patient. However, these Guidelines provide only general recommendations for choosing treatment strategies for colorectal cancer, and they do not control or limit treatment strategies or treatment methods that are not described herein. These Guidelines can also be used as a document to explain the rationale for selecting treatment strategies and treatment methods that differ from those described in these Guidelines.

JSCCR is responsible for the statements in these Guidelines. However, the personnel directly in charge of treatment, not the JSCCR or the Guideline Committee, are responsible for the outcome of treatment.

3. Method used to prepare these Guidelines

(1) Classification of evidence

Levels of evidence were classified as "high-level evidence" or "low-level evidence" as follows:

[High-level evidence]

- Meta-analyses of systematic reviews/randomized controlled trials (RCTs),
- randomized controlled trials,
- nonrandomized controlled trials,
- cohort studies, case–control studies, and cross-sectional studies.

[Low-level evidence]

- Case series studies, case studies, expert opinions, and clinical experience.

(2) Clinical Questions and classification of recommendation categories

As a result of the discussions held by the Guideline Committee, controversial issues were selected as Clinical Questions (CQ), and recommendations were made.

Each recommendation in response to a CQ is accompanied by a classification of the evidence and a classification of recommendation categories based on the consensus reached by the Guideline Committee members. In determining the recommendation categories, in addition to an evaluation of the internal validity of the source of evidence for each recommendation, a comprehensive investigation of the internal validity, external validity, and clinical applicability of each recommendation was performed, considering the following points: (1) the treatment method has a clear scientific rationale and is the best treatment method conceivable; (2) the treatment method is as safe as possible, causes little invasion, and maintains physical function; (3) the treatment method is cost-effective and imposes the smallest financial burden on the patient; and (4) the treatment method is in line with the treatment methods used in actual clinical practice settings in Japan.

Recommendations with which all members of the Guideline Committee agreed were classified as category A or category B recommendations. Recommendations with which three or more members of the Committee disagreed were classified as category D recommendations, and all other recommendations were classified as category C recommendations. The category D recommendations are not included in these Guidelines.

Classification of recommendation categories:

- Category A: unanimous recommendations by the Guideline Committee based on high-level evidence
- Category B: unanimous recommendations by the Guideline Committee based on low-level evidence
- Category C: recommendations that were not agreed to completely by the members of the Guideline Committee, irrespective of the level of evidence
- Category D: recommendations that were not agreed to by three or more members of the Guideline Committee

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Table 1 Number of scientific articles retrieved and selected

	Number of articles retrieved		Number of articles selected		Number of articles retrieved manually
	PubMed	Ichushi	PubMed	Ichushi	
(1) Endoscopic treatment of colorectal cancer	283	214	10	8	8
(2) Treatment of stage 0 to stage III colorectal cancer	347	268	49	11	2
(3) Treatment of stage IV colorectal cancer	189	98	79	14	9
(4) Treatment of liver metastases of colorectal cancer	645	281	255	42	14
(5) Treatment of lung metastases of colorectal cancer	54	134	28	22	2
(6) Treatment of recurrent colorectal cancer	488	125	111	18	7
(7) Adjuvant chemotherapy for colorectal cancer	340	189	154	27	31
(8) Chemotherapy for unresectable colorectal cancer	472	66	234	41	121
(9) Adjuvant radiotherapy for colorectal cancer	398	61	86	6	15
(10) Palliative radiotherapy for colorectal cancer	704	31	107	6	17
(11) Palliative care for colorectal cancer	182	58	19	5	8
(12) Surveillance after surgery for colorectal cancer	1,203	1,213	249	37	13
Total	5,305	2,738	1,381	237	247

4. Literature search

Initially, the literature search was performed for the following 12 broad categories. Then, a further search was done as needed with additional search techniques.

(1) Endoscopic treatment of colorectal cancer
(2) Treatment of stage 0 to stage III colorectal cancer
(3) Treatment of stage IV colorectal cancer
(4) Treatment of liver metastases of colorectal cancer
(5) Treatment of lung metastases of colorectal cancer
(6) Treatment of recurrent colorectal cancer
(7) Adjuvant chemotherapy for colorectal cancer
(8) Chemotherapy for unresectable colorectal cancer
(9) Adjuvant radiotherapy for colorectal cancer
(10) Palliative radiotherapy for colorectal cancer
(11) Palliative care for colorectal cancer
(12) Surveillance after surgery for colorectal cancer

The PubMed and Ichushi-Web databases were selected for the search, and the English and Japanese literature was searched in both databases for the period from January 1983 to December 2007. The task of searching was shared by four members of the medical library; the four members created a search formula by discussion with the Committee members in charge of each item and collected literature during the search period (January 2008 to July 2008). For categories (7) and (8), however, April 2010 was set as the end of the search period. In addition, secondary documents such as UpToDate and literature collected by manual searching were added and critically examined as needed, and other documents such as minutes and guidelines were included as necessary. Of the 8,043 references identified as a result of the searches (5,305 in the PubMed database and 2,738 in the Ichushi-Web database), 1,618 references were retrieved and examined critically (Table 1).

5. Funding

Preparation of these Guidelines was funded by the JSCCR and the Health and Labour Sciences Research Fund (3rd Term Comprehensive 10-Year Strategy for Cancer Control Research Project).

6. Conflicts of interest

None of the members of the committee in charge of the preparation of these Guidelines has any conflict of interest with entities such as any specific profit or nonprofit organizations or any entities related to pharmaceutical or medical products, and the board of the JSCCR confirmed the self-reported absence of any conflicts of interest by the Guideline Committee members.

Treatment guidelines for colorectal cancer

Chapter 1: Treatment strategies for stage 0 to stage III colorectal cancer

1. Endoscopic treatment

General principles underlying the indications for endoscopic resection (Fig. 1)

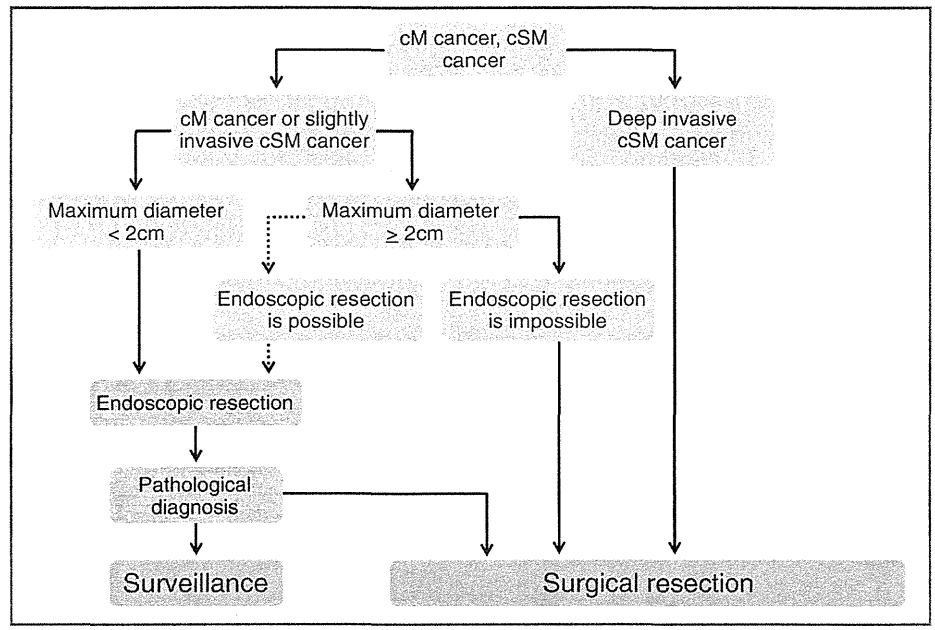

Fig. 1 Treatment strategies for cM cancer and cSM cancer

- There is little possibility of lymph node metastasis, and the size and location of the tumor make en bloc resection possible.

Indication criteria for endoscopic resection:

(1) Intramucosal carcinoma or carcinoma with slight submucosal invasion
(2) Maximum diameter <2 cm
(3) Any macroscopic type

- Endoscopic treatment is a method of endoscopically resecting lesions in the large bowel and of collecting the resected specimens.
- Endoscopic treatment methods consist of polypectomy,[1] endoscopic mucosal resection (EMR),[2] and endoscopic submucosal dissection (ESD).[3]
- In determining the indication for endoscopic treatment and the treatment method, information on the size, predicted depth of invasion, and morphology of the tumor is essential, and the histological type of the tumor should also be taken into consideration.

Comments

- Endoscopic resection is intended for both diagnosis and treatment. It consists of total excisional biopsy in which curability and the need for additional intestinal resection are assessed by histopathological examination of the resected specimens (CQ-1).
- En bloc resection is desirable for accurate diagnosis of the status of carcinoma invasion in the resection margin and the deepest area.
- 2 cm is the largest size of a tumor that can be easily resected en bloc by polypectomy or snare EMR [3] (CQ-2).
- Colorectal ESD has not become a common treatment method, because the technique is difficult and there is a high risk of complications (perforation) [3].
- EMRC (EMR using a cap) involves a high risk of perforation when used for colon lesions.
- If the preoperative diagnosis is intramucosal carcinoma, piecemeal resection can be performed. It should be noted, however, that piecemeal resection is associated with a high incomplete resection rate and a high local recurrence rate [3].

2. Surgical treatment (Fig. 2)

- The extent of lymph node dissection to be performed during colorectal cancer surgery is determined based on the preoperative clinical findings (c) or on the extent of

[1] In polypectomy, a snare is placed on the stalk of the lesion, and the lesion is electrocauterized using a high-frequency current. This method is mainly used for protruding lesions.

[2] In EMR, the lesion is elevated through the local injection of a liquid such as physiological saline into the submucosa, and the lesion is electrocauterized just as in polypectomy. This method comprises the snare method [2] and EMR using a cap (EMRC). It is mainly used for superficial tumors and large sessile lesions.

[3] In ESD, the lesion is elevated through the local injection of a liquid such as sodium hyaluronate solution into the submucosa of the perilesional area; then, circumferential incision of the mucosa surrounding the lesion and dissection of the submucosa are performed with a special knife [3]. ESD is mainly indicated for large tumors that cannot be resected by EMR.

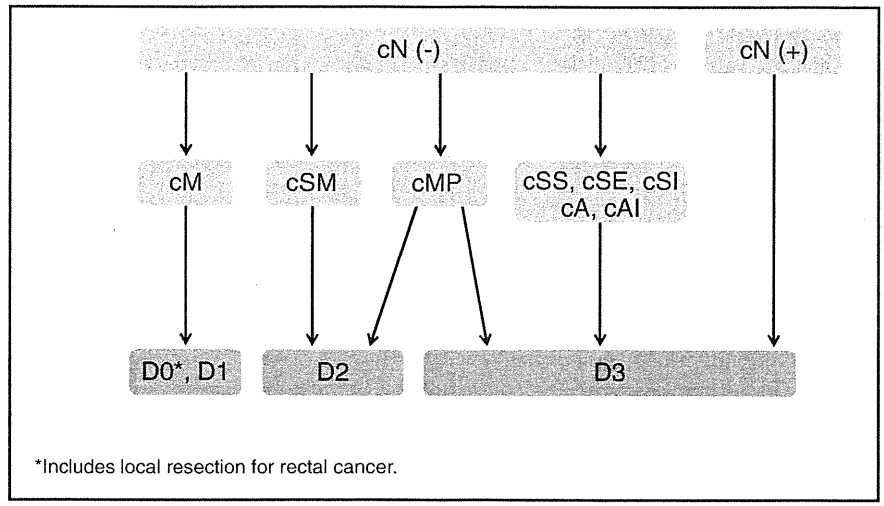

Fig. 2 Surgical treatment strategies for stage 0 to stage III colorectal cancer

lymph node metastasis and depth of wall invasion by the tumor observed intraoperatively (s).

- If lymph node metastasis is suspected based on the preoperative/intraoperative diagnostic findings, D3 dissection is performed.
- If no lymph node metastases are observed based on the preoperative/intraoperative diagnostic findings, lymph node dissection is performed based on the depth of wall invasion by the tumor [4].

(1) Lymph node dissection is unnecessary for M cancer (D0), because M cancer is not accompanied by lymph node metastasis; however, D1 dissection can be performed because the accuracy of the preoperative diagnosis of invasion depth may be insufficient.
(2) D2 dissection is necessary for SM cancer, because the incidence of lymph node metastasis is approximately 10% and because SM cancer is often accompanied by intermediate lymph node metastasis.
(3) Although there is insufficient evidence describing the area of dissection for MP cancer, at the very least D2 dissection is necessary. However, D3 dissection can be performed, because MP cancer is often accompanied by main lymph node metastases and because preoperative diagnosis of depth of invasion is not very accurate.

Surgical treatment of rectal cancer:

- The principle for proctectomy is TME (total mesorectal excision) or TSME (tumor-specific mesorectal excision) [5–8].

[Indications criteria for lateral lymph node dissection]

- Lateral lymph node dissection is indicated when the lower border of the tumor is located distal to the peritoneal reflection and has invaded beyond the muscularis propria [9].

[Local rectal resection]

- Local resection is indicated for cM cancer and cSM cancer (slight invasion) located distal to the second Houston valve (peritoneal reflection). Approaches for local resection are classified into transanal resection, transsphincter resection, and parasacral resection [10]. Transanal resection includes the conventional method in which the tumor is resected under direct vision and transanal endoscopic microsurgery (TEM) [11]. More proximal lesions can be resected by TEM than by the conventional method.

[Autonomic nerve-preserving surgery]

- The autonomic nervous system relating to surgery of rectal cancer consists of the lumbar splanchnic nerves, superior hypogastric plexus, hypogastric nerves, pelvic splanchnic nerves, and the pelvic plexus. Considering factors such as the degree of cancer progression and the presence or absence of macroscopic nerve invasion, preservation of autonomic nerves is attempted in order to preserve urinary and sexual functions as much as possible, provided that curability is unaffected.

Laparoscopic surgery:

- Transabdominal surgery consists of open abdominal surgery and laparoscopic surgery. The indications for laparoscopic surgery are determined by considering the surgeon's experience and skills as well as tumor factors, such as the location and degree of progression of the cancer, and patient factors, such as obesity and history of open abdominal surgery (CQ-3).

Table 2 Lateral lymph node dissection and lateral lymph node metastasis of rectal cancer

	No. of patients	No. of patients who underwent lateral lymph node dissection	Lateral lymph node dissection rate (%)	No. of patients with lateral lymph node metastasis	Lateral lymph node metastasis rate (% of all patients)	Lateral lymph node metastasis rate (% of patients who underwent lateral lymph node dissection)
RS						
sm	124	0	0	0	0.0	0.0
mp	127	6	4.7	0	0.0	0.0
ss/a_1	316	24	7.5	0	0.0	0.0
se/a_2	177	8	4.5	0	0.0	0.0
si/ai	32	14	43.8	1	3.1	7.1
Total	776	52	6.7	1	0.1	1.9
Ra						
sm	138	5	3.6	0	0.0	0.0
mp	149	18	12.1	0	0.0	0.0
ss/a_1	230	58	25.2	4	1.7	6.9
se/a_2	181	59	32.6	7	3.9	11.9
si/ai	15	8	53.3	0	0.0	0.0
Total	713	148	20.8	11	1.5	7.4
RaRb+Rb						
sm	234	37	15.8	2	0.9	5.4
mp	372	218	58.6	20	5.4	9.2
ss/a_1	350	230	65.7	28	7.7	12.2
se/a_2	412	319	77.4	75	18.0	23.5
si/ai	59	48	81.4	17	28.8	35.4
Total	1,427	852	59.7	142	9.8	16.7

Project study by the JSCCR: patients in years 1991–1998

Comments

[Lateral lymph node dissection]

- An analysis of 2916 cases of rectal cancer in the project study by the JSCCR showed that the lateral lymph node metastasis rate in patients whose lower tumor border was located distal to the peritoneal reflection and whose cancer had penetrated through the rectal wall was 20.1% (only patients who underwent lateral lymph node dissection) (Table 2). After performing lateral lymph node dissection for the indication mentioned above, the risk of intrapelvic recurrence decreased by 50%, and the 5-year survival rate improved by 8–9% [9].
- The lateral lymph node metastasis rate of patients whose lower tumor border was located distal to the peritoneal reflection and who had lymph node metastasis in the mesorectum was 27%.
- Urinary function and male sexual function may be impaired after lateral lymph node dissection, even if the autonomic nervous system is completely preserved.

[Aggregate data from the Colorectal Cancer Registry]

- The incidence of lymph node metastasis according to site and depth of invasion, curative resection rate, and 5-year survival rate is shown in Tables 3, 4, and 5 [4].
- The 5-year survival rates after curative resection of stage 0 to stage III colorectal cancer according to site were: all sites 81.3%; colon 83.7%, rectosigmoid 81.2%; Ra–Rb rectum 77.1%.

Chapter 2: Treatment strategies for stage IV colorectal cancer (Fig. 3)

- Stage IV colorectal cancer is associated with synchronous distant metastasis to any of the following organs: liver, lung, peritoneum, brain, distant lymph nodes, or other organs (e.g., bone, adrenal gland, spleen).
- If both the distant metastases and the primary tumor are resectable, curative resection of the primary tumor is performed, and resection of the distant metastases is considered.
- If the distant metastases are resectable but the primary tumor is unresectable, in principle, resection of the primary tumor and distant metastases is not performed, and another treatment method is selected.
- If the distant metastases are unresectable but the primary tumor is resectable, the indication for the resection of the primary tumor is determined, based on the clinical symptoms of the primary tumor and the impact on the prognosis (CQ-4).

Table 3 Incidence of lymph node metastasis according to primary site and depth of invasion

	No. of patients	Extent of lymph node metastasis detected histologically				
		n_0 (%)	n_1 (%)	n_2 (%)	n_3 (%)	n_4 (%)
All sites (C–P)						
sm	2,846	90.1	7.5	2.1	0.1	0.2
mp	3,402	77.0	17.2	4.8	0.7	0.3
ss/a_1	9,862	56.1	27.4	12.2	2.7	1.6
se/a_2	6,175	37.0	32.4	20.2	5.8	4.5
si/ai	1,294	44.0	25.2	15.7	7.6	7.6
Total	23,579	57.6	24.7	12.2	3.2	2.3
Colon (C–S)						
sm	1,757	90.9	6.9	1.9	0.1	0.2
mp	1,598	79.0	16.1	4.4	0.2	0.3
ss/a_1	6,428	57.7	25.8	1.2	2.8	1.4
se/a_2	3,547	38.0	31.7	20.1	5.8	4.4
si/ai	814	46.3	24.8	15.2	5.4	8.2
Total	14,144	58.6	23.8	12.2	3.1	2.3
Rectosigmoid (RS)						
sm	276	90.9	8.0	1.1	0	0
mp	388	78.9	16.2	4.4	0.3	0.3
ss/a_1	1,227	54.9	30.6	10.2	1.6	2.6
se/a_2	793	37.6	36.4	17.9	4.2	3.9
si/ai	134	44.8	28.4	14.2	4.5	8.2
Total	2,818	56.4	28.0	10.9	2.1	2.7
Rectum (Ra–Rb)						
sm	800	88.1	8.6	2.8	0.3	0.3
mp	1,377	74.3	19.0	5.1	1.5	0.2
ss/a_1	2,169	51.7	30.5	13.4	2.8	1.7
se/a_2	1,774	34.7	32.9	21.0	6.3	5.1
si/ai	322	37.6	26.1	17.7	13.7	5.0
Total	6,442	55.7	25.8	12.6	3.7	2.3
Anal canal (P)						
sm	13	84.6	7.7	7.7	0	0
mp	39	69.2	12.8	12.8	2.6	2.6
ss/a_1	38	65.8	18.4	13.2	2.6	0.0
se/a_2	61	42.6	8.2	32.8	14.8	1.6
si/ai	24	45.8	8.3	12.5	16.7	16.7
Total	175	57.1	11.4	19.4	8.6	3.4

National Registry of Patients with Cancer of the Colon and Rectum of the JSCCR: patients in fiscal years 1995–1998. Depth of invasion and the degree of lymph node metastasis were determined according to the rules set forth in the *Japanese Classification of Colorectal Carcinoma* (6th edition)

Comments

- The incidence of synchronous distant metastasis is shown in Table 6.
- Distant metastasis associated with peritoneal dissemination (CQ-5).

(1) Complete resection is desirable for P1.
(2) Complete resection is considered for P2 when easily resectable.
(3) The efficacy of resection of P3 has not been demonstrated.

Chapter 3: Treatment strategies for recurrent colorectal cancer (Fig. 4)

- The goal of treatment for recurrent colorectal cancer is to improve the prognosis and the patient's QOL.
- Treatment methods include surgery, systemic chemotherapy, arterial infusion chemotherapy, thermal coagulation therapy, and radiotherapy.
- An appropriate treatment method is selected with the informed consent of the patient in view of a variety of factors, such as the prognosis, complications, and QOL expected after treatment.

Table 4 Curative resection rate according to stage (lower rows: nos. of patients)

Stage	I	II	IIIa	IIIb	IV	All stages
All patients (C–P)	99.5%	97.0%	91.1%	79.7%	–	78.4%
	5,125	7,168	5,098	2,518	3,953	23,862
Colon (C–S)	99.7%	97.9%	92.2%	82.7%	–	78.1%
	2,838	4,609	2,924	1,436	2,567	14,374
Rectosigmoid (RS)	99.8%	96.2%	91.3%	82.2%	–	77.0%
	548	870	647	258	519	2,842
Rectum (Ra–Rb)	98.9%	95.5%	89.0%	74.7%	–	79.8%
	1,699	1,644	1,497	775	852	6,467
Anal canal (P)	100.0%	80.0%	80.0%	59.2%	–	72.1%
	40	45	30	49	15	179

National Registry of Patients with Cancer of the Colon and Rectum of the JSCCR: patients in fiscal years 1995–1998

Curative resection rate = number of patients with histological curability A cancer/total number of patients who underwent surgery

Staging was performed according to the rules set forth in the *Japanese Classification of Colorectal Carcinoma* (6th edition)

Table 5 Cumulative 5-year survival rate according to site (lower rows: nos. of patients)

Stage	0	I	II	IIIa	IIIb	IV	All stages
Cecum	90.2%	86.7%	81.4%	69.3%	59.5%	9.8%	63.7%
(C)	110	149	252	209	137	225	1,082
Ascending colon	96.3%	90.9%	83.7%	73.9%	57.3%	14.2%	68.3%
(A)	209	257	698	398	254	409	2,225
Transverse colon	94.5%	89.1%	82.6%	70.1%	60.1%	9.6%	67.8%
(T)	176	199	447	270	143	261	1,496
Descending colon	94.7%	90.3%	82.8%	70.9%	57.8%	18.5%	73.4%
(D)	129	151	267	152	67	115	881
Sigmoid colon	95.2%	91.4%	84.5%	81.4%	67.4%	16.6%	75.0%
(S)	559	1,149	1,373	879	394	781	5,135
Rectosigmoid	95.4%	94.6%	79.2%	71.2%	58.1%	11.6%	69.3%
(RS)	184	390	534	448	149	340	2,045
Upper rectum	94.2%	93.1%	77.7%	69.5%	53.7%	9.8%	68.8%
(Ra)	211	471	579	523	238	329	2,351
Lower rectum	92.2%	87.3%	75.2%	60.6%	43.7%	12.3%	66.9%
(Rb)	370	876	653	623	431	336	3,289
Anal canal	91.3%	92.2%	78.9%	43.7%	47.0%	10.2%	59.7%
(P)	12	31	36	32	33	24	168
Colon	94.8%	90.6%	83.6%	76.1%	62.1%	14.3%	71.4%
(C–S)	1,183	1,905	3,037	1,908	995	1,791	10,819
Rectum	92.9%	89.3%	76.4%	64.7%	47.1%	11.1%	67.7%
(Ra–Rb)	581	1,347	1,232	1,146	669	665	5,640
All sites	94.3%	90.6%	81.2%	71.4%	56.0%	13.2%	69.9%
(C–P)	1,960	3,673	4,839	3,534	1,846	2,820	18,672

National Registry of Patients with Cancer of the Colon and Rectum of the JSCCR: patients in fiscal years 1991–1994

Only adenocarcinomas (including mucinous carcinomas and signet-ring cell carcinomas) were counted

Survival rates were calculated by the life table method with death from any cause as an event

Lost to follow-up rate 2%; 5-year censoring rate 19%

Staging was performed according to the rules set forth in the *Japanese Classification of Colorectal Carcinoma* (6th edition)

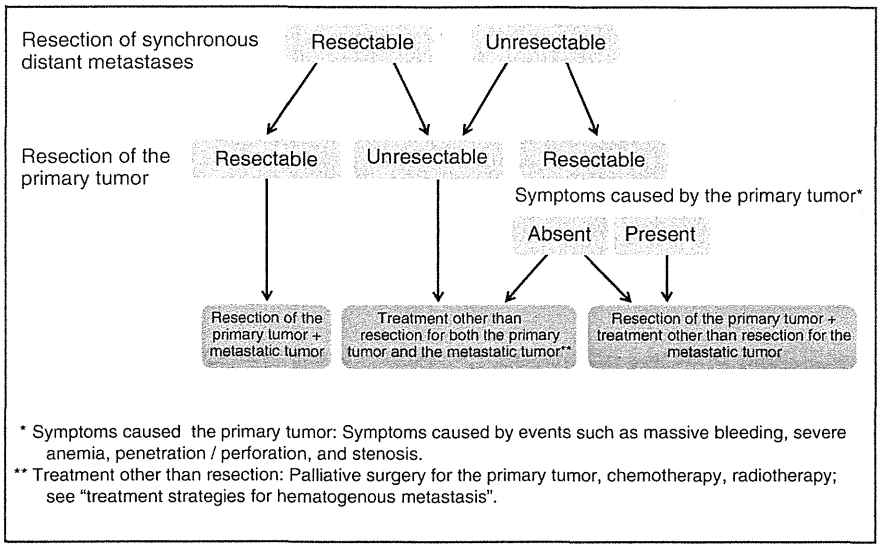

Fig. 3 Treatment strategies for stage IV colorectal cancer

Table 6 Incidence of synchronous distant metastasis of colorectal cancer

	Liver	Lung	Peritoneum	Other sites				
				Bone	Brain	Virchow	Other	Total
Colon cancer	11.4%	1.6%	6.4%	0.3%	0.1%	0.1%	0.4%	0.9%
No. of patients 15,528	1,777	242	993	44	9	19	64	136
Rectal cancer	9.5%	1.7%	3.0%	0.3%	0.1%	0.01%	0.5%	1.0%
No. of patients 10,563	1,002	180	314	36	8	1	57	102
Total no. of patients	10.7%	1.6%	5.0%	0.3%	0.1%	0.1%	0.5%	0.9%
26,091	2,779	422	1,307	80	17	20	121	238

National Registry of Patients with Cancer of the Colon and Rectum of the JSCCR: patients in fiscal years 1995–1998

- If recurrence is observed in a single organ and complete surgical resection of the recurrent tumor(s) is possible, resection is strongly considered.
- If recurrence is observed in more than a single organ, resection can be considered if the recurrent tumors in all of the organs are resectable [12, 13]; however, there is no consensus on the effects of treatment.
- Some authors believe that resection of liver or lung metastases should be performed only after a certain observation period to rule out occult metastases [14].
- Treatment methods for hematogenous metastases (see "Chapter 4: Treatment strategies for hematogenous metastases").

- Local recurrences of rectal cancer take the form of anastomotic recurrences and intrapelvic recurrences.

  (1) Resection is considered for resectable recurrences,
  (2) radiotherapy and systemic chemotherapy, either alone or in combination, are considered for unresectable recurrences.

Comments
[Local recurrence of rectal cancer]

- The extent of spread of the recurrent tumor is evaluated by diagnostic imaging, and resection is considered only for patients in whom complete resection can be expected, after taking into consideration such factors as the pattern of recurrence, symptoms, and physical findings (CQ-6).

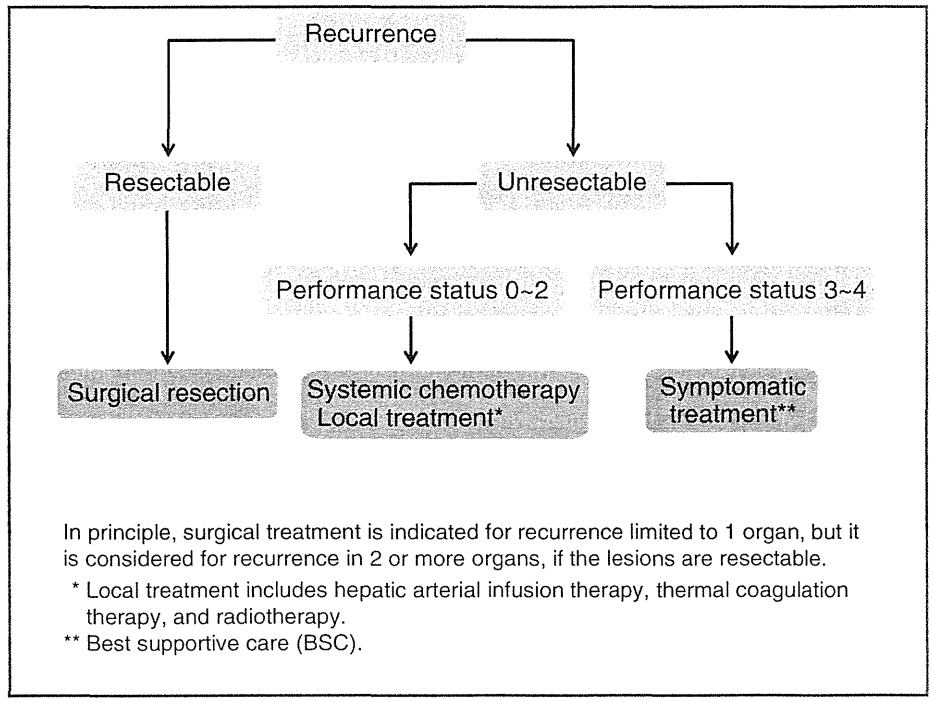

Fig. 4 Treatment strategies for recurrent colorectal cancer

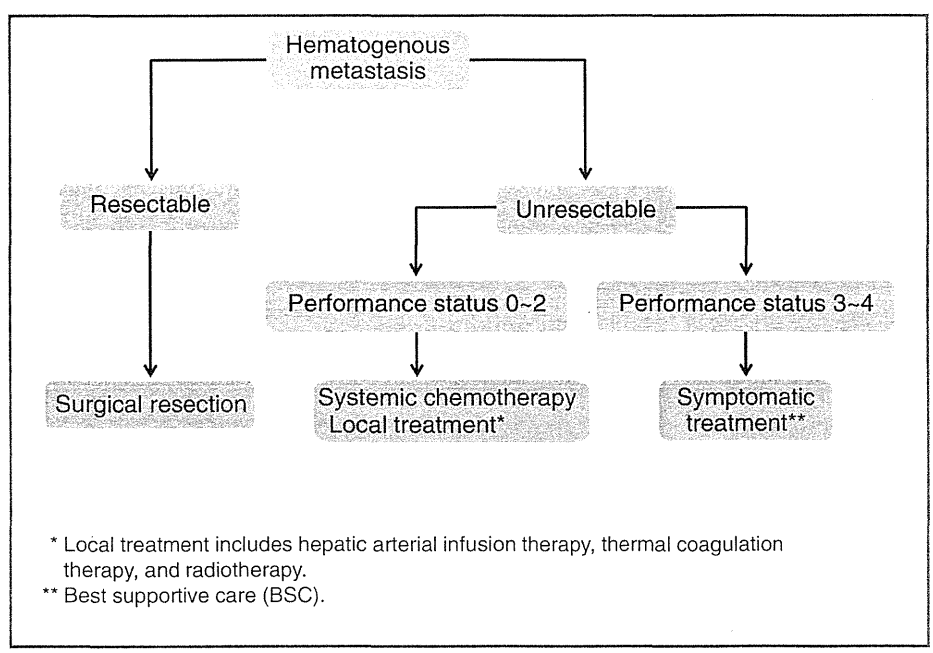

Fig. 5 Treatment strategies for hematogenous metastases

Chapter 4: Treatment strategies for hematogenous metastases (Fig. 5)

1. Treatment strategies for liver metastases

- Treatment of liver metastases is broadly divided into hepatectomy, systemic chemotherapy, hepatic arterial infusion therapy, and thermal coagulation therapy.
- Hepatectomy is recommended for liver metastases when curative resection is possible.
- Hepatectomy consists of systematic resection and partial (nonsystematic) resection.

Indication criteria for hepatectomy

(1) the patient is capable of tolerating surgery,
(2) the primary tumor has been controlled or can be controlled,

(3) the metastatic liver tumor can be completely resected,
(4) there are no extrahepatic metastases or they can be controlled,
(5) the function of the remaining liver will be adequate.

- Systemic chemotherapy and hepatic arterial infusion therapy, either alone or in combination, are considered for patients with unresectable liver metastases whose general condition can be maintained at a certain level or higher (PS 0 to PS 2).
- Thermal coagulation therapy consists of microwave coagulation therapy (MCT) and radiofrequency ablation (RFA).
- If the patient's general condition is poor (PS $\geq$ 3), best supportive care (BSC) is provided.

Comments
[Hepatectomy]

- There are reports showing the efficacy of hepatectomy in patients who have controllable extrahepatic metastases (mainly lung metastases) in addition to liver metastases [12, 13, 15, 16] (CQ-7).
- The efficacy of systemic chemotherapy and hepatic arterial infusion therapy after hepatectomy has not been established (CQ-8).
- The safety of preoperative chemotherapy for resectable liver metastases has not been established (CQ-9).

[Treatment methods other than resection]

- Systemic chemotherapy or hepatic arterial infusion therapy with anticancer drugs is performed alone or in combination for patients with unresectable liver metastases (CQ-10).

2. Treatment strategies for lung metastases

- Treatment of lung metastases consists of pulmonary resection and chemotherapy.
- Pulmonary resection is considered if the metastatic lung tumor is resectable.
- Pulmonary resection consists of systematic resection and partial (nonsystematic) resection.

Indication criteria for pulmonary resection
(1) The patient is capable of tolerating surgery,
(2) the primary tumor has been controlled or can be controlled,
(3) the metastatic lung tumor can be completely resected,
(4) there are no extrapulmonary metastases, or they can be controlled,
(5) the function of the remaining lung will be adequate.

- Systemic chemotherapy is considered for patients with unresectable lung metastases whose general condition can be maintained at a certain level or higher.
- Even if the patient cannot tolerate surgery, stereotactic radiotherapy is considered if the primary tumor and extrapulmonary metastases are controlled or can be controlled and the number of lung metastases is no more than three or four.
- If the patient's general condition is poor, appropriate BSC is provided.

3. Treatment strategies for brain metastases

- Brain metastases are often detected as a part of a systemic disease, and surgical therapy or radiotherapy is considered for lesions in which treatment can be expected to be effective.
- The optimal treatment method is selected after considering the patient's general condition and the status of other metastatic tumors, and evaluating the sizes and locations of metastatic tumors and the number of lesions.
- Radiotherapy is considered for patients with unresectable metastases.

[Surgical therapy]
Indications criteria for removal of brain metastases [17]

(1) The patient has a life expectancy of at least several months,
(2) resection will not cause significant neurologic symptoms,
(3) there are no metastases to other organs, or they can be controlled.

[Radiotherapy]

- The purpose of radiotherapy is to relieve symptoms, such as cranial nerve symptoms and intracranial hypertension symptoms, and to prolong survival time by reducing locoregional relapse.
- Whole-brain radiotherapy is considered for patients with multiple brain metastases and for patients with a solitary brain metastasis for which surgical resection is not indicated.
- Stereotactic irradiation is considered when the number of brain metastases is no more than three or four and the maximum diameter of each metastasis does not exceed 3 cm.

4. Treatment strategies for hematogenous metastases to other organs

- Resection is also considered for other hematogenous metastases, such as to the adrenal glands, skin, and