んの進展様式の1つであり予後因子としても重要な所属リンパ節の切除（郭清）を行う．

しかし，リンパ節転移の可能性がほとんどなく，腫瘍が一括切除できる大きさ（最大径2 cm未満）と部位にある早期がん（粘膜内がん・粘膜下層への軽度浸潤がん）の場合は，内視鏡治療で十分と考えられる．

◉内視鏡治療法

内視鏡治療法にはポリペクトミー，内視鏡的粘膜切除術（EMR）と内視鏡的粘膜下層剥離術（ESD）がある．

ポリペクトミー：病巣茎部にスネアをかけて高周波電流によって焼灼切除する方法であり，主として隆起性病変に用いられる．

EMR：粘膜下層に生理食塩水などを局注して病巣を挙上させ，ポリペクトミーの手技により焼灼切除する方法である．主として表面型腫瘍や大きな無形成病変に用いられる（☞38, 56, 81頁，『疾病と検査』「EMR」）．

ESD：病変周囲，粘膜下層にヒアルロン酸ナトリウム溶液などを局注して病巣を挙上させ，専用の電気ナイフで病変周辺の切開，粘膜下層の剥離を進める手技である．主として，EMRで一括切除できない大きな腫瘍が適応となるが，大腸のESDは手技の難易度が高く，合併症（穿孔）の危険性が高いので，現時点ではまだ一般的な治療法ではない（☞『疾病と検査』「ESD」）．

◉腹腔鏡補助下手術

腹腔鏡補助下手術とは，腹部を大きく開腹し直視下に手術を行う従来法に対して，腹腔鏡とよばれる内視鏡を用いて，二酸化炭素を用いた気腹（気体を腹腔内に充満させること）により手術の作業スペースを作り，細長い鉗子類を操作して，小さな術創でモニターを見ながら手術を行う方法である．この10年で低侵襲手術として大腸がんに対しても急速に普及した．術後の疼痛が軽く，術後の回復が早く，入院期間が短くて済む．また，創が小さいため美容上もすぐれており，術後の合併症の1つである癒着も少ないとされる．手術既往があり癒着が高度の場合や高度肥満の場合は腹腔鏡下手術が困難な場合があり，開腹手術に切り換えることもある．

◉大腸がんの治癒切除率

近年，大腸がんの治癒切除率は向上しているが，それでも約80%に留まる．治癒切除後にも約20%の再発がある．大腸がんの遠隔転移は，肝転移がもっとも多く，初診時において約10%ある．次いで，腹膜転移（5%），肺転移（1.5%），脳・骨・遠隔リンパ節転移など（0.1%未満）となる．同時性遠隔転移巣，ならびに原発巣がともに切除可能な場合には，原発巣の根治切除とともに遠隔転移巣の切除を考慮する．肝転移が切除できた場合の5年生存率は30～50%，肺転移が切除できた場合の5年生存率30～60%であり，切除による治療効果が望める．原発巣にしても遠隔転移巣にしても，根治的な切除不能な場合は全身化学療法が行われるが，局所化学療法，熱凝固療法，放射線療法が行われることもある．遠隔転移巣の切除は不能だが原発巣のみの切除が可能な場合には，臨床症状などから原発巣の切除が考慮される．

◉直腸がんにおける補助療法

直腸がんの手術において，術後の再発抑制や術前の腫瘍量減量，肛門温存を目的とした補助放射線療法が行われるようになってきた．術前に化学療法と組み合わせて放射線化学療法として行われることが多い．

化学療法には，術後再発抑制を目的とした補助化学療法がある．リンパ節郭清を含め，がんの遺残なく切除された症例に対し，予後を改善する目的で術後に実施される全身化学療法である．治療期間は6ヵ月が現時点では標準的であるが，至適投与方法，至適投与期間などに関して，現在多くの臨床試験で検討が行われている．

◉大腸がん手術の実際的手順

大腸がんの手術は次のように行われる．

①開腹（腫瘍の局在に応じた，術野操作の十分可能な皮膚切開）

②腹腔内の検索（腫瘍の局在，大きさ，リンパ節転移の程度，肝臓転移，腹膜播種の有無，他病変の有無などを確認）

③術式の選択（手術前の検査が十分行われていれば，術前に選択した術式と大幅に変わることは少ない）

④腸管の後腹膜よりの脱転（リンパ節郭清を先

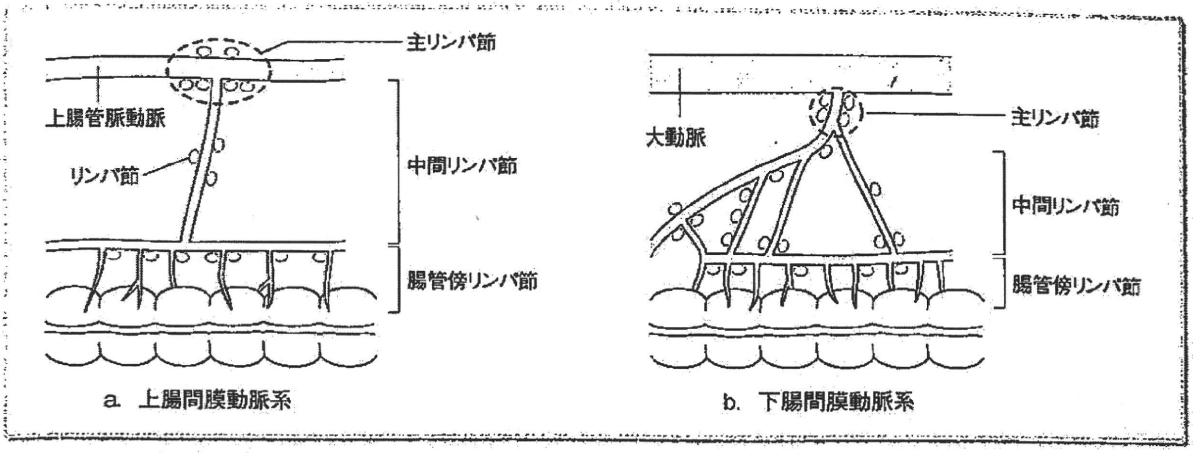

図2　リンパ節分類の基本

[大腸癌取扱い規約，第7版補訂版，大腸癌研究会（編），44頁，金原出版，2009より転載]

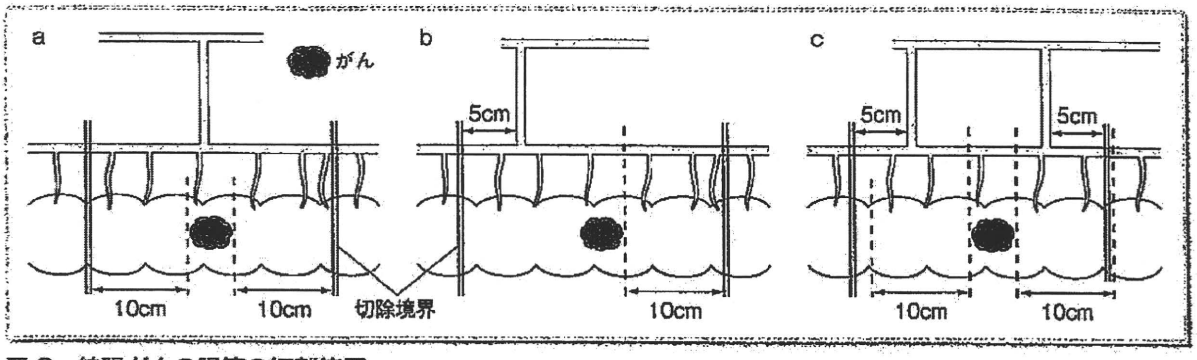

図3　結腸がんの腸管の切離範囲

[大腸癌取扱い規約，第7版補訂版，大腸癌研究会（編），45-46頁，金原出版，2009より改変して転載]

行する場合もある）

⑤リンパ節郭清（血管処理を先行し，リンパ節を含む腸間膜を郭清する）

⑥腸管の切除により，腫瘍と郭清したリンパ節を一続きに摘出

⑦腸管の吻合（人工肛門を造設し，吻合を要さない場合もある）

⑧腹腔内の洗浄，止血，異物の有無の確認（必要であればドレーンの留置）

⑨閉腹

以下に，結腸がん，直腸がん，それぞれの手術方法について具体的に述べる．

1. 結腸がんの手術

◉リンパ節の郭清範囲

リンパ流を考慮し，リンパ節の郭清範囲を決定する．結腸がんのリンパ流は，支配動脈に沿って，その根部へ向かう中枢方向（主リンパ節，中間リンパ節）と，腸管軸に沿った方向（腸管傍リンパ節）の2つの流れがある（図2）．転移リンパ節は支配動脈で囲まれる領域のみに存在し，腸管軸方向では腫瘍から5 cm以内に存在する．したがって，進行結腸がんの3群までの郭清範囲は，中枢方向では主リンパ節と中間リンパ節を，腸管軸方向では，腫瘍より10 cm離しての扇型切除が原則である（図3a）．ただし，腫瘍よりもっとも近い支配動脈より5 cm外側まで切離する（図3b）．腫瘍より10 cm以内に支配動脈が2本ある場合は，それぞれの起始部より5 cm外側を切離する（図3c）．

◉手術法

結腸の支配動脈は，回結腸動脈，右結腸動脈，中結腸動脈，左結腸動脈，S状結腸動脈がある．がん腫の局在場所により，回盲部切除術，（拡大）右半結腸切除術，横行結腸切除術，（拡大）左半結腸切除術，S状結腸切除術などが行われ，切除範囲，吻合法は図4に示したとおりである．結腸は長さが1 m以上もあり，数十cm切

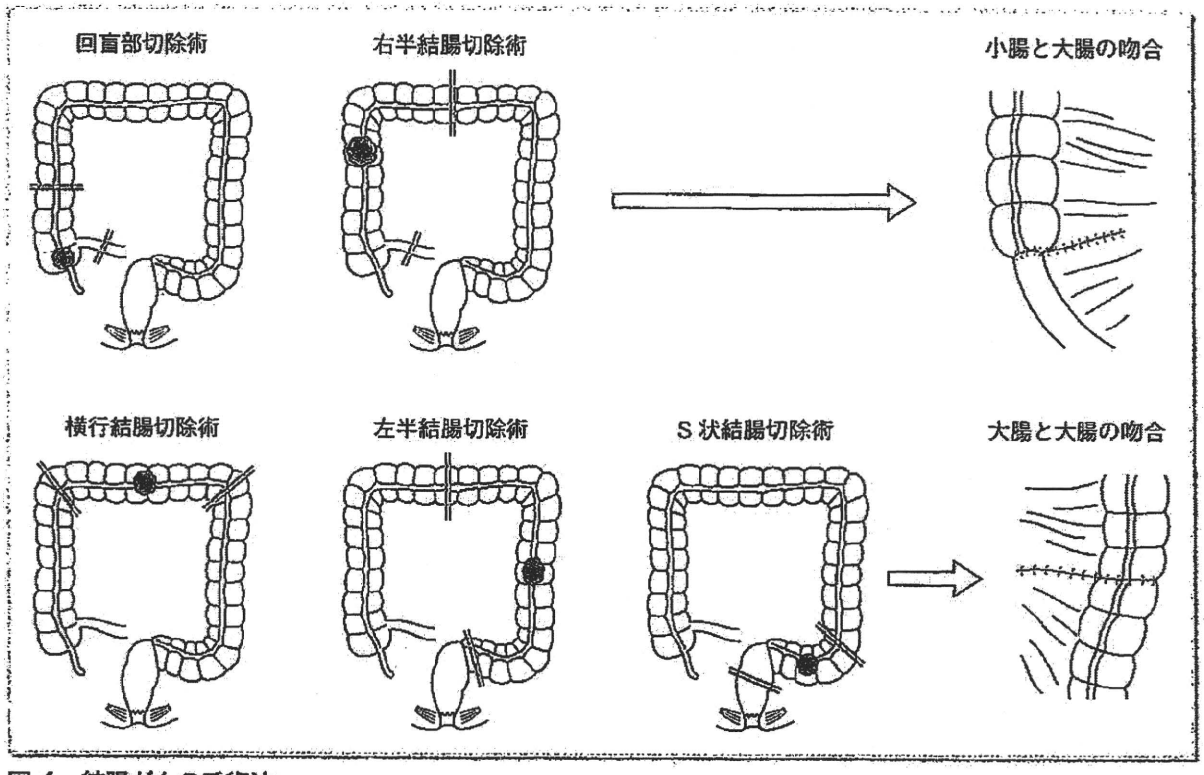

図4　結腸がんの手術法

除しても機能的にはほとんど問題ない．

◉術後の合併症

結腸がんの手術での合併症は少ない．1994年の大腸がん全国登録データによると0.8%である．頻度は，創感染，腸閉塞，縫合不全，出血の順に多いが，そのほか手術共通の合併症としては，肺炎，深部静脈血栓症などがある．

2. 直腸がんの手術

◉リンパ節の郭清範囲

直腸がんのリンパ節の郭清範囲は，図5に示すごとく，中枢方向は下腸間膜動脈根部が主リンパ節，上直腸動脈に沿う領域が中間リンパ節となる．下部直腸がんでは，リンパ流として側方の腸骨動脈領域に向かうもの（主リンパ節に相当）を考慮する必要がある．腸管傍リンパ節としては直腸間膜ごと間膜内のリンパ節を切除すること（直腸間膜全切除）が，局所再発の頻度を下げるために重要である．肛門側の腸管切除は上部直腸がんでは3 cm，下部直腸がんでは2 cmで十分である．口側の切除腸管は，最下S状結腸動脈流入点まで，あるいは10 cmの長いほうを選択すれば十分と考えられる．

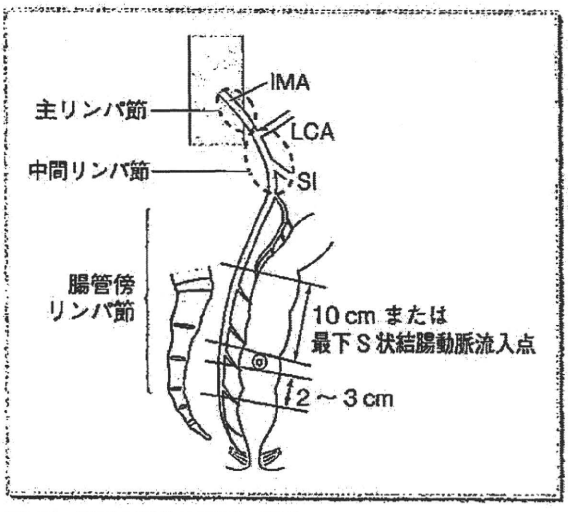

図5　直腸がんのリンパ節
側方リンパ節は下部直腸がんの主リンパ節に相当する．

◉手術法

直腸がんの手術の種類は図6に示すとおりである．腸管の吻合を行って，永久人工肛門を避ける手術を前方切除という（背中側から直腸を切除する方法を後方切除（経仙骨式切除）とよぶのに対応する）．永久人工肛門を造設する手術は，マイルス（Miles）手術（直腸切断術）とハルトマン（Hartmann）手術である．

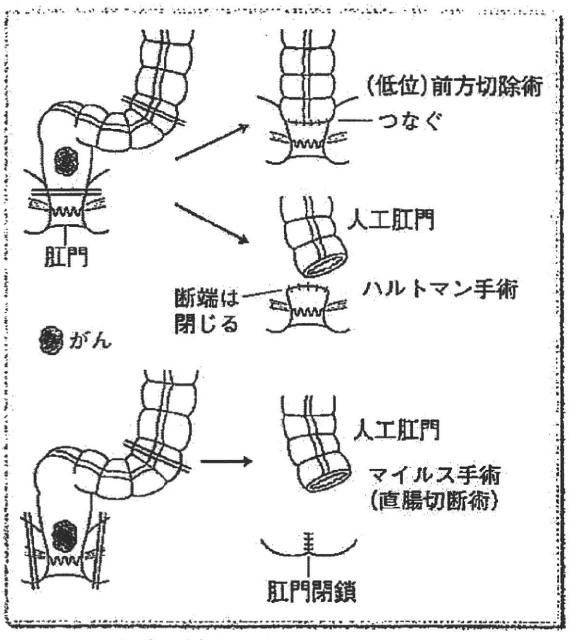

図6 直腸がんの手術法

器械吻合を用いることにより低位で吻合することができるようになり，以前より永久人工肛門造設件数が減っている．前方切除のうち，吻合が腹膜反転部より低位となる場合には低位前方切除術というが，さらに低位の肛門管内で吻合がなされた場合には超低位前方切除とよぶ．一般的ではないが，内肛門括約筋を切除し，肛門に手縫い吻合することにより，さらに低位の直腸がんに対して肛門温存手術ができるようになってきた．吻合が低位になる場合には，術後の縫合不全による再手術を避ける目的で，原発巣手術時に，双口式人工肛門を回腸末端部に造設する場合がある．術後縫合不全のないことが確認された適当な時期（1から6ヵ月後）に人工肛門閉鎖の手術を行う．

◉手術後の機能障害，合併症

手術前後の経過は結腸がんとほぼ同様であるが，合併症はやや多く，とりわけ縫合不全は結腸がんの約10倍となり，吻合が肛門に近づくほど頻度が高くなる．機能的な問題として，排尿障害，排便障害，性機能障害を生じることがある．性機能障害（勃起障害・射精障害），排尿障害（神経因性膀胱）は，自律神経である腰内臓神経から下降する上下腹神経，これに引き続く下腹神経，下部直腸の左右に存在する骨盤神経叢を術中に切除することがあるために生じる．そのほか，直腸が短くなり，便の貯留能がなくなるため，排便回数が増えるという問題点がある．一般的には，肛門に近いところで吻合するほど顕著となり，1日に数十回に及ぶこともある．半年から1年経つと，大概は1日に数回のレベルまで落ち着いてくる．

（冨田 尚裕）

W. 虫垂炎

●虫垂炎とは

虫垂炎（appendicitis）とは，虫垂の非特異的化膿性炎症であり，急性腹症でも頻度の高い疾患である．虫垂の内腔が糞石やX線検査時の造影剤，食物残渣などによって閉塞し，そこに腸内細菌が感染して発症する．処置の遅れにより重篤となる場合があり，素早い対応が要求される．

◉症状

典型的な症状は，食欲不振，心窩部痛，悪心・嘔吐で始まり，数時間経て右下腹部に限局した疼痛へ移行する．37～39℃の発熱を伴うことが多い．一般に，腸管は麻痺性となり排ガス停止がみられることが多い．小児，老人ではこのような典型的症状が出ない場合もある．

◉分類

病理学的特徴により以下の3つのタイプに分類される．

①カタル性虫垂炎：炎症が粘膜層に限局している
②化膿性（蜂巣炎性）虫垂炎：全層に炎症が生じる
③壊疽性虫垂炎：粘膜面や全層に壊死が生じる

これらの中でも，壊疽性虫垂炎は穿孔しやすく重篤化しやすい．

●診断のすすめ方

虫垂炎における診療の流れを図1に示す．

a. 身体所見

虫垂炎に関する圧痛点は多く知られている（図2）．これらのうち，マックバーネー（Mcburney）点とランツ（Lanz）点はとくに診断意義が高く有用である．

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SURGERY TODAY

Review Articles

Strategies for Treating Liver Metastasis from Gastric Cancer

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Abstract
The prognosis of patients with liver metastasis from gastric cancer is dismal. This article reviews the characteristics of gastric cancer metastasizing to the liver, and multimodality of treatments. Differentiated adenocarcinoma, poorly differentiated adenocarcinoma with a medullary growth pattern, and special types, including endocrine carcinoma and hepatoid carcinoma, are likely to metastasize to the liver. The overexpression of growth factors or adhesion molecules is clinically significant for liver metastasis. Surgery for liver metastases arising from gastric adenocarcinoma is reasonable if a complete resection seems feasible after careful preoperative staging. A hepatic resection should always be considered as an option for gastric cancer patients with hepatic metastases. Newer generation cytotoxic agents such as S-1, irinotecan, and taxanes show promising activity for patients with metastases. Adjuvant chemotherapy or molecular targeted therapy will provide significant benefits to patients in the future.

Key words Gastric cancer · Hepatic resection · Liver metastasis · Adjuvant chemotherapy

Introduction

Gastric cancer was the fourth most common malignancy in the world in 2007, with an estimated 1 million new cases.[1] It is the second leading cause of cancer death in men and the fourth among women. In Japan, it is second only to lung cancer among deaths due to cancer.[2] As adequate local control is essential for the treatment of gastric cancer, the standard of care for curable gastric cancer in eastern Asia and the United States is either gastrectomy with D2 lymphadenectomy and without postoperative chemoradiation or D0 or D1 gastrectomy with postoperative chemoradiation.[3–7] However, liver metastasis is found in 4%–14% of patients with primary gastric cancer,[8–11] which is often associated with extrahepatic disease such as peritoneal dissemination, lymph node metastasis, and direct cancer invasion of other organs. Gastric cancer with liver metastasis is a noncurable, fatal disease with a 5-year survival of less than 10%. This article reviews the characteristics of gastric cancer with liver metastasis and the up-to-date treatment of hematogenous metastasis.

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Characteristics of Gastric Cancer with Liver Metastasis

Three histological subtypes of gastric cancer are likely to metastasize to the liver: differentiated adenocarcinoma, poorly differentiated adenocarcinoma, and a special type including endocrine carcinoma and hepatoid carcinoma.[12–15] The differentiated type grows in a papillary or tubular pattern. The poorly differentiated type exhibits a medullary growth pattern. Gastric hepatoid adenocarcinoma is histologically similar to hepatocellular carcinoma.[15] These subtypes have unique characteristics, but share common pathological features such as scant fibrous stroma and abundant tumor blood vessels.[11] The clinicopathological features of gastric cancer with liver metastasis are an expansive pattern of growth, prominent vascular involvement, and a high rate of lymph node metastasis.[16]

Some biological characteristics have been reported to be correlated with liver metastasis. The overexpression of growth factors (c-Met,[17] vascular endothelial growth factor [VEGF][18]) or adhesion molecules (intercellular adhesion molecule 1 [ICAM-1][19] or LFA-3[20]) are clinically significant for liver metastasis.

The *c-Met proto-oncogene* encodes the c-Met receptor, which is a 190-kDa heterodimeric glycoprotein with

two subunits linked by disulfide bonds: a 50-kDa extracellular α-chain and a 145-kDa transmembrane β-chain with a cytoplasmic tyrosine kinase domain.[21] When the ligand of c-Met, hepatocyte growth factor (HGF), binds to c-Met receptor, the tyrosine kinase of the β-chain is activated and the signal is transmitted.[22] c-Met is overexpressed in 18%–68.8% of gastric cancer tissues, and there is a higher degree of c-Met protein expression in carcinoma cells in stage IV gastric cancers with liver metastasis in comparison to that in cancers without liver metastasis.[17] These observations were confirmed at the mRNA level by a semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis. Hepatocyte growth factor is expressed in both carcinoma and stromal cells in gastric cancers.[17] Hepatocyte growth factor produced by cancer cells may induce the proliferative activity of cancer cells in an autocrine fashion. Furthermore, HGF has angiogenic activity that stimulates the proliferation of endothelial cells and is capable of degrading extracellular matrix proteins.[23] The degradation of the basement membrane or extracellular matrix is essential for tumor invasion, and angiogenesis is deeply involved in this process. These observations suggest that HGF may participate in the process of tumor invasion or metastasis through paracrine or autocrine mechanisms. The c-Met/HGF system seems to be more active in the gastric cancer group with liver metastasis.

Vascular endothelial growth factor is a dimeric, heparin-binding glycoprotein that functions as a potent mitogen of vascular endothelial cells, thus providing an opportunity for their migration and organization for the neovascularization of micrometastases.[24] The immunohistochemical expression of VEGF in gastric cancer is associated with increased microvessel density, lymphatic and venous invasion, and lymph node and liver metastases.[18]

Intercellular adhesion molecule-1 and -2 are cell adhesion molecules identified as ligands of lymphocyte function-associated antigen-1 (LFA-1), which is expressed by lymphocytes. These proteins are expressed by various cells, such as vascular endothelial cells, fibroblasts, and epithelial cells.[25–27] Intercellular adhesion molecule-1 is a glycoprotein with an extracellular region that has an immunoglobulin-like structure and, thus belongs to the immunoglobulin superfamily.[28] Its expression is enhanced by cytokines such as interleukin (IL)-1 and interferon (IFN)-γ.[27] Intercellular adhesion molecule-1 is a cell adhesion molecule that takes part in the destruction of cancer cells by immunocytes. Overexpressed ICAM-1 may be released from cells in a local cancer cell nest and enter the serum as soluble ICAM-1 (s-ICAM-1), which suppresses immunocytes by binding to LFA-1. The rate of ICAM-1 expression increases slightly according to the stage and its expression is higher in advanced cancer.[19] In addition, the rate of ICAM-1 expression is markedly correlated with metastasis to the lymph nodes and the liver. ICAM-1 is overexpressed in cancer cells and released as s-ICAM-1, which promotes hematogenous metastasis by suppressing local anticancer immunity. The serum s-ICAM-1 level may be useful for monitoring hematogenous metastasis during postoperative follow-up, and the development of an absorption technique for s-ICAM-1 may reduce postoperative hematogenous metastasis.

Intercellular adhesion molecule-1 and LFA-3 are adhesion molecules and members of the immunoglobulin superfamily that appear to be essential for the interactions of T cells with other immune cells and their targets by mediating strong adhesion.[29] A higher percentage of lymphocytes in hepatic sinusoids in normal livers express LFA-1, MAC-1, ICAM-1, and LFA-3 on their surface than peripheral blood lymphocytes,[30] and both ICAM-1 and LFA-3 are strongly expressed in hepatocytes and other target structures from patients with inflammatory liver diseases.[31] Primary tumors and metastases in draining lymph nodes demonstrate a broad range of LFA-3 expression. In contrast, distant metastases (liver and peritoneum) have uniformly high frequencies of LFA-3-positive cells, thus suggesting a selective advantage for these cells in the establishment of distant metastases.[20]

The molecular mechanism of liver metastasis still remains essentially unknown. Experimental analyses of liver metastasis using gastric cancer cell lines or animal models are therefore important to reveal the mechanism of hematogenous metastasis and to develop new therapeutic strategy. Most gastric cancer cell lines were derived from ascites or lymph node metastasis, and there are cell lines derived from liver metastasis.[32,33] Both genetic alterations and cellular adjustments to the microenvironment are required for hepatic metastasis in gastric cancer.[34] The parental YCC-16 shows multiple metastases, whereas the liver metastatic clones metastasize to the liver only. In vertebrates, dystroglycan is generated from a single gene (DAG1), which is located on the chromosome 3p. YCC-16 presents the lowest DAG1 expression level while the cell line from the orthotopic primary tumor (S1L0) presents the highest. The DAG1 expression level in the liver metastatic clones increases gradually with passages.

Surgery for Liver Metastasis

A surgical resection of liver metastasis from gastric cancer is rarely indicated, because liver metastasis is often associated with extrahepatic disease, such as peritoneal dissemination, lymph node metastasis, and direct

Table 1. Results of hepatic resections for metastasis from gastric cancer

First author[Ref]	Year	No. of pts. with liver metastasis	No. of pts. who underwent hepatic resection	Median survival time (months)	5-Year survival after resection	Prognostic factors by multivariate analysis	Indications for surgery
Ambiru[40]	2001	—	40	12	18%	Synchronous metastasis	Metachronous metastasis
Okano[9]	2002	90	19 (21.1%)	21	34%	Multiple metastases Synchronous metastasis	Solitary metastasis Metachronous metastasis
Shirabe[36]	2003	—	36	NA	26%	Vessel invasion Number of metastasis	No vessel invasion One or two metastases
Sakamoto[11]	2007	182	37 (20.3%)	31	11%	Bilobar metastasis Tumor diameter ≥4 cm	Unilobar metastasis Tumor diameter <4 cm
Koga[38]	2007	247	42 (17.0%)	34	42%	Multiple metastases Serosal invasion	Solitary metastasis No serosal invasion
Cheon[41]	2008	58	22[a] (37.9%)	17	23%	Multiple metastases ($P = 0.0519$)	Solitary metastasis

NA, not assessed
[a] Patients who underwent a combined curative resection of gastric cancers and hepatic metastases

cancer invasion of other organs.[13] In contrast to colorectal cancer, the vast majority of patients with gastric cancer and liver involvement may reflect generalized disease. Selected patients accounting for one-fifth of all cases with liver metastasis can undergo hepatic resection[11] (Table 1). The survival rate after hepatectomy is rather unsatisfactory, because two-thirds of the patients develop intrahepatic recurrence.[11] This high recurrence rate within 2 years of the surgery might suggest the presence of occult intrahepatic metastases even at the time of the hepatectomy. There have so far been few reports of a repeat hepatectomy resulting in favorable outcomes.[10]

The significant prognostic factors are the stage of the primary gastric cancer, number of liver metastases, timing of the hepatectomy, and the surgical margin.[9,11,35–40] Ochiai et al.[37] suggested that a hepatic resection should be attempted in patients with synchronous or metachronous metastases if there is no serosal invasion by the primary gastric tumor, and if the primary tumor has neither venous nor lymphatic invasion in the case of metachronous metastases. Solitary metastases from gastric cancer are recommended for surgical treatment.[9,38] Sakamoto et al.[11] noted that unilobar metastasis and/or tumors less than 4 cm in diameter may be indicated for surgical resection. Furthermore, synchronous metastasis is not a contraindication for hepatectomy. As for surgical margin, some[39,40] concluded that positive surgical margins should be avoided, and others[11,41] reported that an extensive safety resection margin may not be essential for better outcomes of hepatic resection in gastric cancer. Cheon et al.[41] reported that the survival rates after curative intent do not differ between curative and palliative resections. At present, surgery for liver metastases arising from gastric adenocarcinoma is reasonable if a complete resection seems feasible after careful preoperative staging. A hepatic resection should be considered as an option for gastric cancer patients with hepatic metastasis.

Recurrent tumors usually develop in the liver following a hepatic resection for gastric metastases (62%–79%),[11,41] thus indicating that the remaining liver should be a focus for postoperative monitoring. A sensible strategy for improving survival would be close observation for a second relapse in the liver and adjuvant chemotherapy after surgery. The efficacy of adjuvant chemotherapy after resection of liver metastases has not been fully evaluated. A second hepatic resection is not usually selected for most recurrent intrahepatic metastases but systemic chemotherapy may be administered.[7]

Radiofrequency Ablation

Radiofrequency ablation (RFA) is a popular alternative to surgery for tumor ablation due to its safety, availability, and wide applicability to primary or secondary hepatic malignancies.[42,43] Yamakado et al.[44] reported a prospective study that evaluated the efficacy of hepatic arterial infusion chemotherapy (HAIC) with use of an implanted port followed by radiofrequency (RF) ablation for the treatment of liver metastasis of gastric cancer. Seven patients without extrahepatic metastasis were enrolled. The maximum tumor size was less than 3 cm in one patient and 3.2–6.0 cm in the other six patients (mean, 4.4 ± 1.5 cm). The maximum tumor size was reduced to 3 cm or less (mean, 2.4 ± 0.4 cm; $P < 0.03$) after HAIC in all patients. Radiofrequency ablation was performed for all residual liver tumors, resulting in

complete tumor necrosis, with a median survival time of 16.5 months. The complementary role of the radiofrequency is recommended in the palliative treatment of the hepatic metastases of advanced gastric cancer that are difficult to treat surgically.[45] The size of the hepatic metastasis is the most important factor in determining whether complete local ablation can be achieved.[43] In general, lesions measuring less than 2.5 cm in diameter have a greater than 90% chance of being destroyed, and less than 50% of tumors measuring greater than 5 cm are likely to be completely ablated.[42] Gannon and Curley[46] recommended not treating tumors >5 cm in maximal diameter with RFA. With improvements in ablation techniques and instruments, the number and extent of RFA treatments is increasing.[47] However, the efficacy, indications, and limitations of this therapy for liver metastasis from gastric adenocarcinomas have not yet been studied in a large series of patients.

Systemic Chemotherapy for Liver Metastasis

The standard treatment regimen for patients with unresectable gastric cancer was a matter of debate for a long time. S-1 (Taiho Pharmaceutical, Tokyo, Japan) is an oral anticancer drug that combines tegafur, a prodrug of fluorouracil, with 5-chloro-2,4-dihydropyrimidine (CDHP), and potassium oxonate in a molar ratio of 1:0.4:1.[48] Phase II studies of S-1 have yielded responses of 44%–54% in patients with advanced gastric cancer,[49,50] and in Japan, S-1 is mainly used as the first-line treatment for this type of cancer. The response rates for liver metastasis in these phase II studies are 25%–31%.[49,50] A phase III study conducted by the Japan Clinical Oncology Group (JCOG), study 9912, revealed that S-1 alone is no worse than fluorouracil alone.[51] In this study, irinotecan plus cisplatin was no better than fluorouracil alone. However, in subgroup analyses, the effect of irinotecan plus cisplatin on progression-free survival and overall survival was greater in patients with target lesions, such as lymph node metastases or liver metastases, than in those without target lesions. In addition, a trial of S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial) verified that the median overall survival was significantly longer in patients assigned to S-1 plus cisplatin (13.0 months) than in those assigned to S-1 alone (11.0 months).[51] This phase III trial identified S-1 plus cisplatin as one of the standard first regimen for advanced gastric cancer in Japan. In exploratory subgroup analyses using a Cox proportional-hazards model, the effect of S-1 plus cisplatin on overall survival was greater in patients with peritoneal metastasis than in those without peritoneal metastasis, and also in patients without target tumors than in those with target tumors.[52] A randomized phase III study of S-1 plus irinotecan versus S-1 alone (GC0301/TOP-002)[53] failed to prove the superiority of S-1 plus irinotecan to S-1 alone. The subgroup analysis of this study has not been disclosed. A phase III study of docetaxel and S-1 versus S-1 (JACCRO GC-03) is ongoing to determine the optimal combination.[54] A phase II study of docetaxel and S-1 combination therapy revealed that the response to docetaxel-S-1 was not affected by the type of organs involved or the histologic tumor type. The highest overall response rates among the metastatic sites were observed for liver (64.7%), locoregional lymph node (60.0%), and peritoneum (60.0%).[55] Newer generation cytotoxic agents such as S-1, irinotecan, and taxanes show promising activity for patients with metastatic gastric cancer. These agents will likely be evaluated in the future for their role as adjuvant and neoadjuvant therapy. The systemic or local control of the disease may give patients various chances to undergo curative surgery.

Elsewhere, triplet therapies are standard, such as docetaxel and cisplatin plus fluorouracil (DCF) in the United States,[56] or epirubicin and cisplatin plus fluorouracil (ECF) or epirubicin and oxaliplatin plus capecitabine (EOX) in Europe.[57] The efficacy of these therapies for liver metastases has not been reported. In Korea, cisplatin plus oral capecitabine (XP) is also reported to be recommended for advanced gastric cancer.[58]

Hepatic Arterial Infusion

Regional hepatic arterial infusion (HAI) of chemotherapy takes advantage of the first-pass effects of cytotoxic agents, delivering higher local drug concentration to unresectable liver tumors with fewer significant systemic side effects.[59] There have been few reports of hepatic arterial infusion for patients with liver metastases of gastric cancer.[60–63] In 1990s the response rate of HAI of MMC and cisplatin was 73% (17/23), and the median survival period was 11.8 months.[60] In an earlier preliminary phase II study performed by Arai et al.,[61] a high response rate of 73.3% (22 of 30 cases) was achieved by HAI therapy using 5-fluorouracil (5-FU), doxorubicin and mitomycin-C (MMC; FAM regimen), or 5-FU, epirubicin, and MMC (FEM regimen) in patients with hepatic metastases of gastric cancer. The multicenter phase II study evaluated the efficacy of the FEM regimen showed a response rate of 55.6% (35/63) and the mean 50% survival was 10.5 months.[63] However, most responders died due to the progression of extrahepatic lesions.

To enhance the effectiveness of regional treatment in patients with liver carcinoma, cytotoxic drugs may be

combined with alternative therapeutic strategies such as partial vascular blockade using degradable starch microspheres (DSM).[64] When DSM combined with a cytotoxic drug are infused through the hepatic artery, the steep drug concentration gradient to the tumor tissue results in higher tissue drug concentrations which may elicit an increased antitumor response. Hirasawa et al.[65] reported the effects of transcatheter arterial chemoembolization (TACE) using DSM in patients with hepatic metastases from gastric cancer after prior systemic chemotherapy. Infusion of epirubicin hydrochloride (40–70 mg/body) following arterial chemoembolization with DSM and mitomycin C (4–12 mg/body) was administered. The response rate was 62.5% (5/8) and the median survival time was 36.1 months. After the progression of the disease following systemic chemotherapy, HAI is another treatment for the patients with liver metastasis only.

Tumor Markers

The prevalence of positive tumor markers among gastric cancer patients selected for surgical resection is low, and when positive, provides little prognostic value.[66,67] The commonly used markers in gastric cancer are carcinoembryonic antigen (CEA)[68] and sialyl Lewis^a antigen (CA19-9).[69] A high percentage of CEA-positive tumors are noted in differentiated gastric cancers.[70] The preoperative level of CEA is strongly correlated with clinical estimation of tumor mass and progression of the disease.[71] Ishigami et al.[72] preoperatively estimated the levels of CEA and CA19-9 in patients with gastric cancer. The rates of CEA (≥5 ng/ml) and CA19-9 (≥37 U/ml) were 19.5% and 18%, respectively. The level of serum CEA and CA19-9 significantly correlated with depth of invasion, hepatic metastasis, and curativity. CA19-9 may be especially useful as a marker for peritoneal recurrence of gastric cancer, and CEA for recurrence to the liver.[73] Korenaga et al.[74] reported that CEA doubling time predicts life expectancy in patients with adenocarcinoma of the gastrointestinal tract. Positive CEA suggests recurrence to the liver. An RT-PCR analysis of CEA mRNA in the peripheral blood seems to be a promising tool for the early detection of micrometastatic circulating tumor cells in gastric carcinoma patients.[75]

There are some reports that α-fetoprotein (AFP)-producing gastric cancers are associated with a poor prognosis with lymphatic and venous microinvasion of the gastric wall, and high rates of liver metastasis, of both the synchronous and metachronous types.[76,77] There is limited information on the cellular or molecular characteristics of AFP-producing gastric cancers. c-Met overexpression is more frequently observed in AFP-producing gastric cancers than those not producing AFP.[78] The c-Met proto-oncogene encodes the c-Met receptor which regulates cell proliferation or migration,[79] and HGF has been identified as its ligand.[80] α-Fetoprotein-producing gastric cancer cells with higher c-Met expression grow more progressively in response to HGF, which is abundantly produced within cancer tissue. α-Fetoprotein-producing gastric cancers have a high proliferative activity, weak apoptosis, and rich neovascularization.[81] α-Fetoprotein-producing gastric cancers arise as an aggressive clone with extensive loss of heterozygosity (LOH) and high fractional allelic loss.[82] For informative cases, LOH is most frequently detected on 17p (100%), followed by 13q (88%), 3p (87%), 5q and 9p (80%), 11q (70%), 18q (58%), 16q (53%), and 8p (50%). The presence of heterogeneous patterns of LOH suggests that the AFP-producing carcinoma foci might evolve through genetic progression and/or genetic divergence. It is interesting to note that the loci of 13q that are commonly deleted in AFP-producing gastric carcinoma are also frequently deleted in hepatocellular carcinoma, which often presents with raised serum AFP values.

Sialyl Lewis^a (CA19-9) and sialyl Lewis^x antigens (SLX) may play a role in tumor metastasis by serving as functional ligands in the cell adhesion system.[80] An elevated preoperative serum SLX level is a predictor of poor outcome after a resection for gastric cancer, and a logistic regression analysis revealed that a high serum SLX level is an independent predictor of liver metastasis.[83]

Sialyl Tn antigen (STN) is a cell-membrane-bound mucin-like carbohydrate structure that is sometimes expressed in solid tumors because of blocked synthesis of the core carbohydrate chain of mucin-like structures.[84] Preoperative high serum levels of STN predict both liver metastasis and poor prognosis after a resection for gastric cancer.[85,86]

Gastric cancer metastasized to the liver is found to overexpress HER2 at a significantly higher incidence (65%) than primary gastric cancers (38%).[87] All these gastric cancer liver metastasis cell lines are highly sensitive to gefitinib, a specific inhibitor of EGFR tyrosine kinase (Iressa) rather than anti-HER2 antibody trastuzumab (Herceptin), whereas most of the HER2 low-expressing lines are not. The antitumor effect of gefitinib is due to the effective inhibition of HER2-driven constitutive activation of phosphatidylinositol-3-kinase (PI3K)/Akt pathway, and the acquired resistance to gefitinib is due to the constitutive activation of the Ras/MAPK pathway in compensation for the PI3K/Akt pathway.[87] Gastric cancer liver metastasis with HER2 overexpression could be a potential molecular target for gefitinib and trastuzumab.

Conclusions and Future Prospects

The optimal treatment of gastric cancer with liver metastases without peritoneal dissemination or other distant metastases remains a matter for debate. Surgery for liver metastases from gastric cancer may be indicated if a complete resection seems feasible after careful preoperative staging. Synchronous metastasis is therefore not a contraindication for a hepatectomy.

The preliminary results of a large neoadjuvant chemotherapy study have demonstrated the efficacy of this approach with tumor downstaging and increase in the curative R0 resection rate. Major advances in the treatment of gastric cancer have occurred during the past several years and have improved the care of patients with this form of tumor.

An improved understanding of the biological characteristics, such as the expression of growth factors or adhesion molecules including signal pathways in gastric cancer, will assist in the development of new targeted therapies and perhaps best define those patients with potentially chemosensitive tumors. Therefore, multimodality therapies and strategies are necessary for patients with liver metastases.

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The impact of a high-frequency microsatellite instability phenotype on the tumor location-related genetic differences in colorectal cancer

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Abstract The purpose of this study was to evaluate the genetic background of colorectal cancer according to the tumor site, and to investigate the impact of the genetic features regarding the lesion location of colorectal cancer. Microsatellite instability (MSI), DNA index, and the mutation and loss of heterozygosity of the *TP53* gene were systemically examined in 180 Japanese colorectal cancer cases. The correlation between these genetic features and clinicopathologic factors was analyzed. A logistic regression was undertaken to analyze the association between genetic features and tumor locations. The data demonstrated location-related genetic differences in colorectal cancer. The proximal subset was distinct in patterns of genomic instability and *TP53* gene defects. The genetic features of distal colon cancers paralleled those of rectal cancers. Intriguingly, a multivariate analysis implicated MSI as the only factor significantly associated with tumor location. When MSI tumors were excluded, the statistical association between tumor location and alternations in the DNA index and *TP53* vanished. The location-related differences of colorectal cancer were derived from the unequal distribution of the MSI tumors. On the other hand, the microsatellite stable colorectal cancers were genetically homogeneous regardless of the tumor location. Therefore, instead of tumor location, microsatellite status should be a major focus for the study of colorectal cancers in the future.

1. Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related death in Western developed countries, and ranks the first when smoking-related cancers are excluded. According to the GLOBOCAN database (http://www.iarc.fr/), the incidence of CRC in Japan has dramatically increased during the past 60 years. The age-standardized rate of CRC in Japan is similar to that in North American and Western European countries [1,2].

The colonic and rectal subdivision of CRC is simple and practical, thus is applied by most cancer database registries [3]. On the other hand, many studies have indicated that cancers from the proximal and distal subset of the large bowel, separated by the splenic flexure, differ in genetic characteristics and clinicopathologic features [4]. Therefore, it is crucial to determine whether the tumor location of CRC should be a major concern, which will profoundly affect both clinical practice and the field of research.

Genomic instability is a defining characteristic of most human cancers [5]. Two patterns of genetic aberrations, chromosomal instability (CIN) and microsatellite instability (MSI), reveal the existence of at least two independent pathways for the tumorigenesis of CRC [6]. A high frequency MSI (MSI-H), which is derived from aberrations in the DNA mismatch repair system, occurs in about 15% percent of CRC [7]. The MSI-H cancers usually have a diploid or near-diploid chromosomal content and are predisposed to mutations at the nucleotide level at two to three orders of magnitude greater than normal cells [8]. The remaining majority of CRC cells bear the CIN phenotype, which is characterized by a greatly increased rate in the gain/loss of a whole chromosome or a large fraction of chromosomes, although the mechanisms underlying CIN remain largely unknown [9]. The alternation of the DNA index (DI) reflects an overall change in the chromosome. The DI, which was accessed by laser scanning cytometry (LSC), was suggested to be strongly associated with the

Dr. Oki and Dr. Zhao designed the project, planned the experiments and designed the methods; Dr. Ando was involved in the analyses and experiments; Dr. Morita, Dr. Kakeji and Prof. Maehara contributed to the presentation, interpretation and discussion of the results obtained in article form.

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chromosomal aberrations detected by fluorescent in situ hybridization [10].

The *p53* pathway is responsible for a variety of intrinsic and extrinsic stress signals that impact cellular homeostatic mechanisms that monitor DNA replication, chromosome segregation, and cell division alternations [11]. A *TP53* gene defect is one of the most common genetic alternations in CRC as well as many malignancies [12]. Classically, a mutation and a loss of heterozygosity (LOH), i.e., the "two hits," of *TP53* finally lead to the occurrence of a defect in the p53-related pathways [13].

One interesting study suggests that the inclusion of MSI tumors account for the location-related difference of colon cancer [14]. The study estimated the p53 defects by immunohistochemical staining. This concept was herein examined by standard methodologies in a series of Japanese patients. Rectal cancer was also included in the study panel.

2. Materials and methods

2.1. Patient selection and sample preparation

This study enrolled 180 Japanese patients diagnosed to have CRC who underwent surgery without neoadjuvant chemotherapy in the Department of Surgery II at Kyushu University Hospital from 1994 to 2003. Written informed consent for the study of excised tissue was obtained from each patient. The entities of the proximal colon (cecum, ascending colon, and transverse colon), distal colon (descending and sigmoid colon), and rectum were defined by separation of the splenic flexure and height of the promontorium.

DNA was extracted from cancerous tissue specimens and the corresponding noncancerous mucosa specimens [15]. The remaining specimens were routinely processed for the histopathologic analysis and diagnosis.

2.2. Microsatellite analysis for MSI and LOH detection

High-resolution fluorescent microsatellite analysis has been described in detail elsewhere [15]. Briefly, genomic DNA isolated from cancerous and corresponding noncancerous tissue specimens was used to amplify microsatellite loci by polymerase chain reaction (PCR) using primer sets labeled with a fluorescent compound, ROX (6-carboxy-x-rhodamine) or HEX (6-carboxy -20,40,70,4,7, -hexachloro-fluorescein). The fluorescently labeled PCR products were mixed, denatured, and loaded onto an ABI 310 sequencer (Applied Biosystems, Foster City, CA) for fragment analysis. The data were processed using the GeneScan software package (Applied Biosystems).

An alteration in the length of a microsatellite PCR fragment from cancerous tissues was MSI positive. MSI is defined by the frequency of positive findings of five reference markers [16]. MSI status is classified as follows: microsatellite instability high (MSI-H), more than 30% of loci demonstrate MSI; microsatellite instability low, 30% or less loci demonstrates MSI; and microsatellite stability, no MSI detected. MSI-H is labeled "MSI (+)" and the rest "MSI (−)"[16].

D17S796 and D17S1353 were used as markers for *TP53* gene LOH detection. LOH was defined as the presence of heterozygous peak heights in more than 30% of alleles in either of the loci tested [17]. If the two clusters overlapped in their electrophoresis profile, the case was not informative (NI) regarding LOH estimation.

2.3. Determination of the DNA Index (DI)

LSC (CompuCyte Corporation, Westwood, MA) was used to detect the chromosomal DNA content; i.e., DI as described [18]. In brief, cell nuclei were recovered from two pieces of a 50 μm-thick slice from paraffin-embedded blocks that had a tumor area greater than 30% in dimension. Single-layered nuclei were spread on a slide glass, stained with propidium iodide/RNase A (Sigma, St. Louis, MO), covered, and observed. A DNA content histogram was generated, and DI was calculated according to previously published guidelines [19]. The DI of lymph cell nuclei with dimensions of about 40 μm^2 was used as the reference (DI = 1.0). Tumors with a single peak of DI < 1.2 were defined as diploid or, otherwise, as aneuploid [20].

2.4. TP53 gene mutational analysis

TP53 exons 5–9, including exon–intron junctions, were amplified by PCR using "p53 primers" (Nippon Gene,

Table 1
Clinicopathological backgrounds of proximal and distal colon cancer

	Location		
	Proximal	Distal	*P*
Age (mean)	66.2	62.7	0.119
Gender			
Male	33	28	0.900
Female	26	21	
Gross			
Polypoid	49	43	0.493
Flat	10	6	
Histologic grade			
Grade 1	28	28	0.293
Grade 2	18	18	
Grade 3	13	3	
pStage			
I–II	29	21	0.514
III–IV	30	28	
Lymphocyte infiltration[a]			
Negative	31	24	0.736
Positive	26	23	
Lymphatic invasion[b]	2	2	
None	33	33	0.185
Present	25	15	

[a] Four missing data.
[b] Two missing data.

Tokyo, Japan) and *Ex Taq* DNA polymerase with 3' exonuclease activity (TaKaRa Bio Inc.). The PCR products were purified and used as templates for cycle sequencing reactions with the Big Dye Terminator Cycle Sequencing Kit (version 1.0; Applied Biosystems). The mutations found in a PCR product were verified by reverse sequencing and then were reconfirmed in two independently amplified PCR products.

2.5. Statistical analysis

Statistical analyses were performed using the SAS software package (version 9.0; SAS Institute, Cary, NC). Student's *t*-test was used to compare the average ages of the patients. The chi-square test and Fisher's exact test were used to examine relationships between categoric variables and the genetic markers.

Due to the similar genetic backgrounds, as determined by a univariate analysis, distal and rectal cancers were combined. Logistic regression models were used to estimate the multivariable relationship between the alternations of these genetic markers and the odds of tumor locations. Initial logistic regression models examined the main effects only. Interactions between these factors were explored in subsequent logistic models. All reported P values were two-sided, and $P < 0.05$ was considered statistically significant.

3. Results

The clinicopathologic backgrounds of the tumors used in this study are shown in Table 1. The four key genetic factors induced in the current study have all been well documented, though they are seldom studied in the same large panel. The MSI, DNA aneuploidy, *TP53* gene mutation, and *TP53* LOH were detected in 14.4, 73.3, 36.1, and 59.4% percent of cases, respectively, and these findings did not differ from those reported in Caucasians.

The association between genetic features and clinicopathologic variables are shown in Table 2. The genetic factors were not associated with age, gender, pathologic gross type, tumor stage, or lymphatic invasion. The tumor location was found to be significantly associated with all the four markers studied. Moreover, MSI was associated with a lower degree of differentiation and less frequent

Table 2
Genetic features and clinicopathologic variables in CRC

	MSI			DNA index			*TP53* gene			*TP53* LOH[h]		
Factors	MSI(−)	MSI(+)	*P*	Diploidy	Aneuploidy	*P*	WT[f]	Mut[g]	*P*	LOH(−)	LOH(+)	*P*
Age (mean)	64.4	65.7	0.585	65.0	64.4	0.758	63.6	66.3	0.124	65.9	63.8	0.289
Gender												
Male	89	15	0.992	26	78	0.555	64	40	0.442	29	63	0.710
Female	65	11		22	54		51	25		23	44	
Location												
Proximal colon	41	18	<0.001	23	36	0.034	45	14	0.047	24	28	0.045
Distal colon	44	5		11	38		29	20		11	30	
Rectum	69	3		14	58		41	31		17	49	
Gross												
Polypoid	135	21	0.360	39	117	0.211	96	60	0.082	43	94	0.384
Flat	19	5		9	15		19	5		9	13	
Histologic grade												
Grade 1[a]	76	11	<0.001	26	61	0.440	58	29	<0.001	28	50	0.509
Grade 2[b]	65	5		15	55		36	34		16	43	
Grade 3[c]	13	10		7	16		21	2		8	14	
TNM stage												
I–II	67	14	0.328	27	54	0.068	55	26	0.310	25	46	0.545
III–IV	87	12		21	78		60	39		27	61	
Lymphocyte infiltration[d]												
Not prominent	70	19	0.006	31	58	0.005	60	29	0.265	31	49	0.073
Prominent	80	6		14	72		51	35		19	56	
Lymphatic invasion[e]												
None	91	15	0.835	29	77	0.726	66	40	0.682	31	64	0.961
Present	61	11		18	54		47	25		20	42	

P values showing statistical significance are underlined.
[a] Well differentiated.
[b] Moderately differentiated.
[c] Poorly differentiated or undifferentiated.
[d] Five missing data.
[e] Two missing data.
[f] *TP53* wild type.
[g] *TP53* mutation.
[h] 21 cases were not informative for *TP53* LOH estimation.

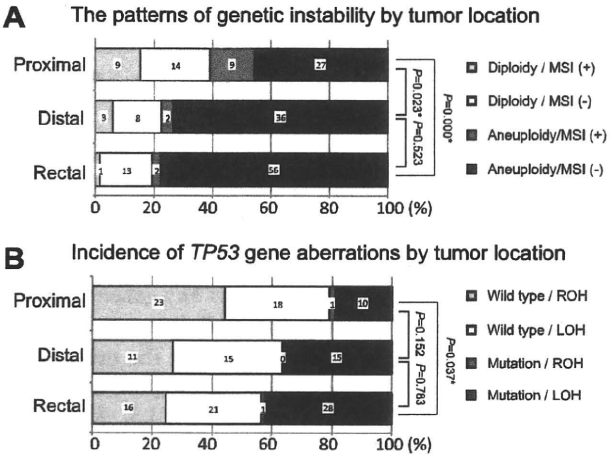

Fig. 1. The location-related genetic differences of CRC. MSI (+), high-frequency microsatellite instability; MSI (−), low-frequency microsatellite instability and microsatellite stability; WT, *TP53* wild type; Mut, mutated *TP53*; LOH (−), retention of heterozygosity; LOH (+), loss of heterozygosity. *A total of 11 cases were not informative for LOH estimation and thus were treated as missing data for the analysis. (A) Schematic illustration of the genetic instability patterns by tumor location. (B) Incidence of*TP53* gene aberrations by tumor location.

lymphocyte infiltration, which is inconsistent with previous reports [21]. The *TP53* mutations were associated with higher histologic grade, and the *TP53* LOH tended to be associated with lymphocyte infiltration.

The patterns of genomic instability were schematized according to DNA ploidy and MSI by tumor locations (Fig. 1A). The aneuploidy/MSI (−) and diploidy/MSI (+) suggested the classic CIN and MSI tumorigenesis pathways. The patterns of genetic instability were distinct in proximal colon cancers.

The integration of the *TP53* mutation and LOH was compared by tumor locations (Fig. 1B). The incidences of mutation and LOH were significantly different between the proximal colon cancer and the cancers from other parts of the colon and rectum. The tumor location showed no statistical association with either the *TP53* mutational spectra or special amino acid changes (data not shown).

The four genetic factors were associated with each other on the basis of the chi square test findings (Table 3). An acquired logistic fit model was applied successively to analyze the association between tumor location and genetic features (Table 4). The tumor location was inducted as the outcome variable, and genetic features as the explanatory variables. According to the similar genetic features of the distal colon and rectal cancers, they were combined into one group for analysis. MSI was the only parameter independently associated with tumor location ($P = 0.001$). The logistic models considered the interactions between the factors and revealed an identical result, thus only the model considered to be the main effect was shown.

To further demonstrate the involvement of MSI to tumor site–related differences, the 26 MSI (+) tumors were excluded, and then the associations were re-analyzed (Table 5, A and B). As expected, the associations between the clinicopathologic variables and DI, *TP53* mutation, and LOH vanished.

4. Discussion

It is crucial to determine whether the tumor location defines distinct genetic features of CRC because of its potential significance in both the clinical and research fields [22]. In this study, four genetic markers — MSI, DI, *TP53* mutation, and LOH — were investigated in a series of Japanese CRC to analyze whether any association exists between the tumor location and these crucial genetic markers.

The aberrations of these factors were inconsistent with most previous studies in Caucasians, despite the reported ethical difference of the CRC genetics [23]. The proximal subsets of colon cancer manifested distinct features in the genetic factors studied; i.e., less DI alternation, high MSI, low *TP53* mutation, and LOH. In addition, from a histopathological standpoint, the proximal colon cancers were prone to be poorly differentiated and less prominent in lymphocyte infiltration (data not shown), which is inconsistent with a previous study [24]. The distal colon cancer and rectal cancer shared similar genetic and biologic features.

Intriguingly, the multivariate analysis indicated that MSI was the only factor significantly associated with tumor

Table 3
Genetic features of sporadic CRC

		MSI			DNA index			*TP53* gene		
Factors		MSI (−)	MSI (+)	*P*	Diploidy	Aneuploidy	*P*	WT	Mut	*P*
DNA index	Diploidy	35	13	0.006						
	Aneuploidy	119	13							
TP53 gene	WT[a]	90	25	<0.001	47	68	<0.001			
	Mut[b]	64	1		1	64				
TP53 LOH	LOH(-)	37	15	0.004	40	12	<0.001	50	2	<0.001
	LOH(+)	96	11		2	105		54	53	

P values showing statistical significance are underlined.
[a] *TP53* wild type.
[b] *TP53* mutation.

Table 4
Logistic regression analysis of genetic factors and the distal versus proximal localization of CRC

	Object	Control	Odds ratio	95% CI[c]	*P*
CIN[a]	Aneuploidy	Diploidy	1.437	0.326–6.334	0.632
MSI[b]	MSI (−)	MSI (+)	5.052	1.915–13.325	0.001
TP53 LOH	LOH	ROH	1.701	0.401–7.217	0.471
TP53 mutation	Mutation	Wild type	1.170	0.480–2.848	0.730

P value showing statistical significance is underlined.
[a] Chromosomal instability.
[b] Microsatellite instability.
[c] 95% confidence interval.

location, which was similar to a previous report on colon cancer in a Caucasian population [14]. This report estimated the p53 changes by immunohistochemical staining, and MSI were studied by a denaturing gel electrophoresis–autoradiograph assay, which is supposed to have unavoidable technological problems [25].

In the current study, MSI and LOH were studied by an accurate fragment analysis [15], and the *TP53* mutation was evaluated by direct sequencing. The DI was detected by LSC, a methodology that enables a direct morphologic evaluation of the cell population. Furthermore, when MSI-H tumors were excluded, the associations between the pathologic variables and the genetic features disappeared. The analysis strongly indicated that the location-related differences of CRC were caused by the inclusion of MSI-H tumors.

The MSI phenotype is a derived genetic or epigenetic defect in the DNA mismatch repair machinery, which plays a crucial role in DNA metabolism and cell cycle control [26]. The MSI defines a tumorigenesis pathway characterized by an elevated mutation rate in simple-repeat nucleotides independent of CIN. The less frequent DI aberrations in the proximal colon could be explained by the inclusion of the MSI tumors. MSI tumors comprise

Table 5A
Genetic features and clinicopathologic factors in MSI (+) tumors

	DNA index			*TP53* gene			*TP53* LOH[h]		
Factors	Euploidy	Aneuploidy	*P*	WT[f]	Mut[g]	*P*	LOH(−)	LOH(+)	*P*
Age (mean)	66.0	63.2	0.478	64.3	66.6	0.665	65.0	64.1	0.819
Gender									
Male	12	15	0.228	22	5	0.695	14	13	0.485
Female	13	8		18	3		13	8	
Location									
Proximal	16	12	0.489	25	3	0.415	17	11	0.302
Distal	5	4		7	2		6	3	
Rectal	4	7		8	3		4	7	
Gross									
Polypoid	2	4	0.326	6	0	0.242	2	4	0.226
Flat	23	19		34	8		25	17	
Histologic grade									
Grade 1[a]	11	12	0.321	17	6	0.233	12	11	0.531
Grade 2[b]	9	4		12	1		9	4	
Grade 3[c]	5	7		11	1		6	6	
pStage									
I–II	16	11	0.259	22	5	0.696	17	10	0.288
III–IV	9	12		18	3		10	11	
Lymphocyte infiltration[d]									
Negative	18	13	0.113	26	5	1.000	20	11	0.063
Positive	5	10		12	3		5	10	
Lymphatic invasion[e]									
None	14	16	0.422	24	6	0.46	15	15	0.375
Present	10	7		15	2		11	6	

[a] Well differentiated.
[b] Moderately differentiated.
[c] Poorly differentiated or undifferentiated.
[d] Two missing data.
[e] One missing data.
[f] *TP53* wild type.
[g] *TP53* mutation.
[h] A total of 21 cases were not informative for *TP53* LOH estimation.

Table 5B
Genetic features and clinicopathologic variables in MSI (−) tumors

Factors	DNA index			*TP53* gene			*TP53* LOH[h]		
	Diploidy	Aneuploidy	*P*	WT[f]	Mut[g]	*P*	LOH(−)	LOH(+)	*P*
Age (mean)	63.6	64.6	0.629	63.0	66.4	0.053	65.6	68.3	0.388
Gender									
Male	19	70	0.634	49	40	0.318	20	57	0.578
Female	16	49		41	24		17	39	
Location									
Proximal colon	14	27	0.140	28	13	0.320	14	20	0.115
Distal colon	8	36		24	20		7	29	
Rectum	13	56		38	31		16	47	
Gross									
Polypoid	28	107	0.135	76	59	0.140	30	86	0.203
Flat	7	12		14	5		7	10	
Histologic grade									
Grade 1[a]	21	55	0.312	48	28	0.191	22	45	0.418
Grade 2[b]	11	54		31	34		12	42	
Grade 3[c]	3	10		11	2		3	9	
TNM stage									
I–II	19	48	0.102	42	25	0.220	16	41	0.956
III–IV	16	71		48	39		21	55	
Lymphocyte infiltration[d]									
Not prominent	20	50	0.069	42	28	0.642	18	43	0.664
Prominent	13	67		45	35		18	51	
Lymphatic invasion[e]									
None	22	69	0.511	52	39	0.819	23	57	0.683
Present	12	49		36	25		13	38	

[a] Well differentiated.
[b] Moderately differentiated.
[c] Poorly differentiated or undifferentiated.
[d] Five missing data.
[e] Two missing data.
[f] *TP53* wild type.
[g] *TP53* mutation.
[h] A total of 21 cases were not informative for *TP53* LOH estimation.

a pathologically and clinically distinct subtype of CRC, which tend to occur in the proximal subset of the colon, and show poor differentiation, mucin production, and Crohn's-like peritumoral lymphoid response [27].

In CRC, MSI is exclusive to the *TP53* mutation, which indicates a distinct tumorigenesis pathway independent of the defects in the p53-related pathways. The MSS and MSI-L were similar in *TP53* aberrations and DI alternations (data not shown), thus suggesting a similar, if not identical, tumorigenesis background. Therefore, they were combined as MSI (−). The proximal accumulation of MSI tumors might thus demonstrate a relatively low incidence of *TP53* aberrations.

The prognostic value and clinical application of MSI as a chemosensitivity indicator still remains controversial [28]. To some extent, the diversity of the biologic significance of the MSI phenotype might be attributed to the lack of a standardized methodology for MSI detection [29]. MSI is implied as a favorable prognostic factor, according to a detailed review of 2006 ASCO [30]. Another report concluded that *TP53* mutations in proximal tumors are associated with a significantly worse survival, based on a univariate analysis, but the significance was diminished by a multivariate analysis when other factors, including MSI, were considered [31]. The current results can explain such a finding; i.e., the favorable prognosis of the *TP53* wild type tumors in the proximal subset of the colon were probably accounted for by the MSI-H tumors, which have a better prognosis and are possibly *TP53* wild type. Similarly, another study suggests that *TP53* mutations, when considered with tumor sites, are associated with a poor prognosis [12]. MSI, however, was not considered in that study. The different prognosis effects of *TP53* mutation by tumors locations may be partly derived from the inclusion of MSI-H tumors. Therefore, MSI is a molecular marker that can provide valuable information in colon cancer patients. In the appropriate clinical setting, MSI data can be used in clinical decision-making. Specifically, the favorable outcome of stage II colon cancers with MSI indicates that such patients should not receive adjuvant chemotherapy.

The mutational events of some important oncogene/tumor suppressor genes such as *KRAS*, *APC*, *PIK3CA*, and *BRAF* were also studied. The alternations of these genes did not show any obvious location-related differences, so they were not documented. These indicated that

the lower digestive tract cancers share significant genetic features, however, despite the location-related differences that are emphasized in this study.

To sum up, proximal colon cancers were genetically distinct from distal colon cancers and rectal cancers; the rectal cancers and distal colon cancers share similar genetic profiles. Importantly, MSI was the major and only factor associated with the CRC site–related genetic and clinicopathologic differences. MSI (−) tumors had similar genetic profiles, regardless of the tumor locations. This study proposes that site-related differences in CRC might be attributable to the inclusion of MSI tumors. These findings support the idea that MSI indicates a unique category of CRC. The current study strongly implies that the MSI status, instead of the tumor location, should thus be a major concern in the understanding of CRC.

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High expression of BUBR1 is one of the factors for inducing DNA aneuploidy and progression in gastric cancer

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Gastric cancers show high frequency of DNA aneuploidy, a phenotype of chromosomal instability. It is suggested that the abnormal spindle assembly checkpoint is involved in DNA aneuploidy, but the underlying mechanism is still unclear. We studied the mechanism by assessing the expression of BUBR1 in gastric cancer. The DNA ploidy patterns of 116 gastric cancer samples obtained from the Department of Surgery and Science at Kyushu University Hospital were analyzed. Of those, DNA aneuploidy was seen in 70 (60.3%) cases of gastric cancer. The expression of BUBR1 was studied by immunohistochemistry in 181 gastric cancer samples and by real-time RT-PCR in several gastric cancer cell lines. Ninety-one (50.3%) cases had high expression of BUBR1 and those cases correlated significantly with DNA aneuploidy ($P < 0.05$). Also high expression of BUBR1 cases had significant correlation with deep invasion, lymph node metastasis, liver metastasis, and poor prognosis. In gastric cancer cell lines, high expression of BUBR1 had a significant relationship with DNA aneuploidy ($P < 0.05$). Then, gastric cancer cell lines MKN-28 and SNU-1 were transfected with full-length BUBR1 to observe the significance of the change in BUBR1 expression. Enforced expression of BUBR1 resulted in changes to the ploidy pattern and high Ki-67 expression. Collectively, our clinical and *in vitro* data indicate that high expression of BUBR1 may be one of causative factors for the induction of DNA aneuploidy and progression of gastric cancer. (*Cancer Sci* 2010; 101: 639–645)

DNA aneuploidy is a state of cells with an abnormal number of chromosomes. More than a century ago, David Paul Hansemann observed that cancer cells have abnormal chromosome numbers.[1] In 1997, Lengauer *et al.* reported that DNA aneuploidy was seen in 85% of colorectal cancers.[2] This form of chromosomal instability reflected a continuing cellular defect that persisted throughout the lifetime of the cancer cell and was independent from microsatellite instability which was a recessive trait. DNA aneuploidy is an important phenomenon for cancer cells; however, whether or not DNA aneuploidy may be a cause for carcinogenesis is still controversial. To date many analyses have focused on DNA aneuploidy and it is now known that mutation or inactivation in p53, 'the guardian of genome', results in DNA aneuploidy.[3,4]

Recently, it has been defined that DNA aneuploidy occurs due to disorders in the spindle assembly checkpoint.[5] This checkpoint is the mechanism which delays the separation of sister chromatids until all the kinetochores of chromosomes are correctly attached to the spindle.[6] It has been reported that knockdown or overexpression of spindle assembly checkpoint molecules resulted in DNA aneuploidy and carcinogenesis in mice.[7] The disorders in spindle assembly checkpoint molecules may be involved in DNA aneuploidy and carcinogenesis in humans.

BUBR1 kinase, a member of the BUB (budding uninhibited by benzimidazole) gene family, is one of the key molecules in the spindle assembly checkpoint. It accumulates on the unattached kinetochore.[8] BUBR1 targets Cdc20, an APC/C (anaphase promoting complex/cyclosome; E3 ubiquitin ligase) activator, and prevents the premature onset of anaphase.[9,10] The degradation of Cdc20 represents a critical control mechanism ensuring the inactivation of APC/C in response to the spindle assembly checkpoint.[11] In addition, BUBR1 independently interacts with securin.[12] Matsuura *et al.* reported that a mutation in the *BUBR1* gene is detected in premature chromatid separation (PCS) syndrome, which has been often associated with chromosomal instability and malignancies. Furthermore, an abnormal spindle assembly checkpoint was observed in this syndrome.[13,14]

Although mutations of BUBR1 have been rarely observed in clinical samples,[15,16] high expression of BUBR1 has often been reported in several malignancies and correlated with chromosomal instability,[17–19] but not thus far in gastric cancer.

Herein, we report the significance of DNA aneuploidy and BUBR1 high expression in gastric cancer by analyzing gastric cancer clinical samples and cell lines.

Material and Methods

Patients studied. This study included 181 unselected Japanese patients with primary gastric cancer, all of whom underwent a gastrectomy between 1994 and 2006 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University Hospital, Fukuoka. They included 121 men and 60 women, ranging in age from 29 to 90 years (mean, 64.2 years). In each case, a careful informed consent was obtained. Those who refused were not included. A thorough histological examination was made by using H&E-stained tissue preparations, and the histological classification was made according to the general rules set up by the Japanese Gastric Cancer Association.[20] No patient treated preoperatively with cytotoxic drugs was included in this study.

Immunohistochemical staining of BUBR1. Formalin-fixed, paraffin-embedded tissue specimens were used for immunohistochemical staining. A paraffin block which contained both cancerous tissue, invading the deepest area of the stomach wall, and adjacent noncancerous tissue, was used in each case. Immunohistochemical staining was done as described in previous reports.[19,21,22] Briefly, the sections were pretreated with

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