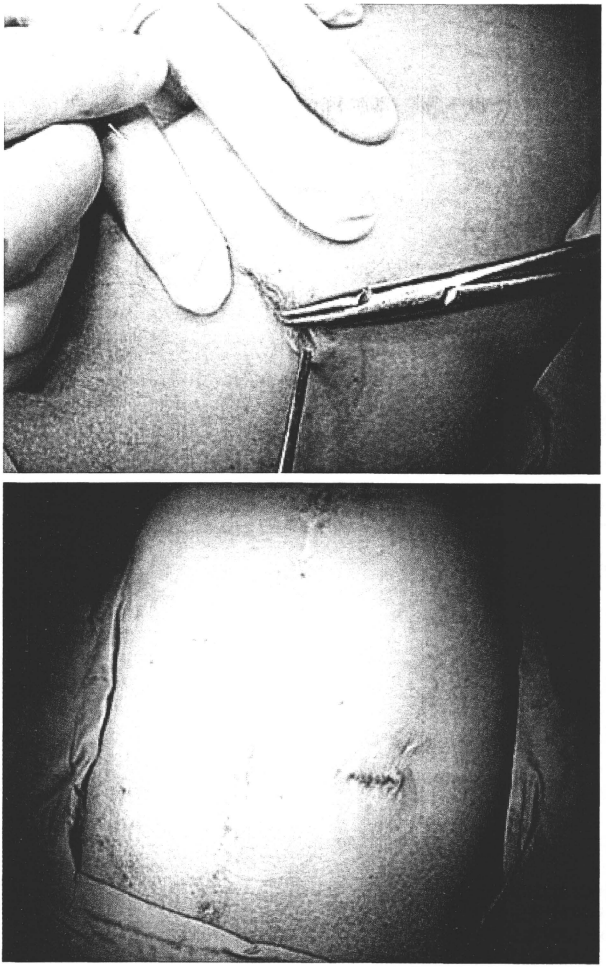

図７　小開腹併用腹腔鏡下手術の手術創
結果的に小開腹創１カ所のみからの操作で終了した

## まとめ

イレウス管により減圧が効いている循環動態が安定した症例，保存的治療で軽快するが再発を繰り返す症例が癒着性イレウスに対する腹腔鏡下手術のよい適応と考える。腹腔鏡下手術は低侵襲ということで積極的に行われ始めたが，腹腔内の状況確認が困難な場合がある。イレウスの原因をはっきりと確認できない場合や腸管損傷などの副損傷が疑われる場合は，小開腹併用または開腹手術に移行して確実な対処を行うことが重要である。

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特集 外科臨床に必要な漢方治療の知識

外科系臨床雑誌

# 外科治療 12

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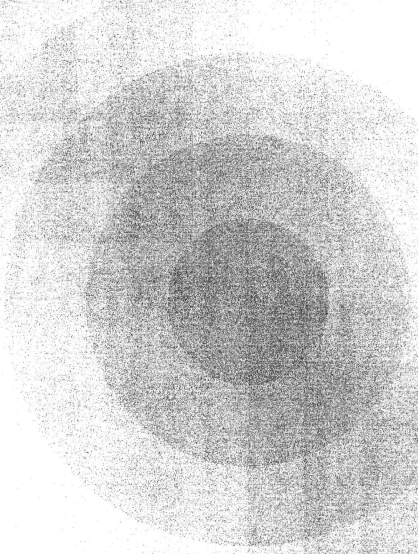
## 特集 外科臨床に必要な漢方治療の知識



- 今日の問題
- 内視鏡手術
- 臨　　床
- 臨床の実際
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2011年 2 月号特集予告　癌の治療成績ー日本と欧米の相違ー

内視鏡手術

# 右側結腸癌に対する腹腔鏡手術のコツ

*Surgical technique of laparoscopic surgery for right side colon cancer*

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右側結腸癌に対する腹腔鏡下手術は，**授動操作**と**リンパ節郭清**が重要である．授動操作は十二指腸前面が透見できる薄い膜から剝離を開始し（modified medial approach），外側・尾側・頭側に十分剝離を進めることで腸間膜が自然に授動され，後の外側剝離が容易になる．リンパ節郭清はSurgical trunkの分枝状況や動脈との関係をあらかじめ把握し，腹膜や組織を少しずつ丁寧に剝離していくことが重要である．新しいアプローチとして単孔式腹腔鏡下手術やロボット支援腹腔鏡下手術が登場してきた．

## はじめに

大腸癌に対する腹腔鏡下手術は低侵襲手術として認知されただけでなく，進行癌に対する安全性を検証した臨床試験により直腸癌や横行結腸癌を除く大腸癌に対しては標準治療となってきた[1)-3)]．右側結腸癌に対する腹腔鏡下手術は，血管系のバリエーションが多彩なだけでなく，血流豊富な静脈系のリンパ節郭清が中心となることで難易度が高い手術と位置づけられている．右側結腸癌に対する腹腔鏡下手術に関して，安全に手術を進めるために行っているわれわれの工夫を，**授動操作**と**リンパ節郭清**について示す．

また，近年新しいアプローチとして単孔式腹腔鏡下手術やロボット支援腹腔鏡下手術が注目を浴びている．大腸癌に対する応用もすでに始まっており，これらに関する現状も報告する．

## I. 手術手技上の工夫

右側結腸癌に対する腹腔鏡下手術操作は，**授動操作**と**リンパ節郭清**が重要なポイントとなる．これらの操作を行ううえでわれわれが行っている工

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*Key words*：右側結腸癌／腹腔鏡下手術／単孔式腹腔鏡下手術／ロボット支援腹腔鏡下手術

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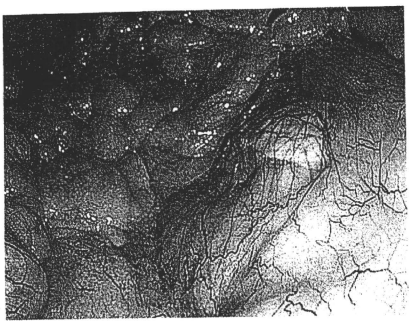

図1 十二指腸・膵臓が透見できる薄い膜

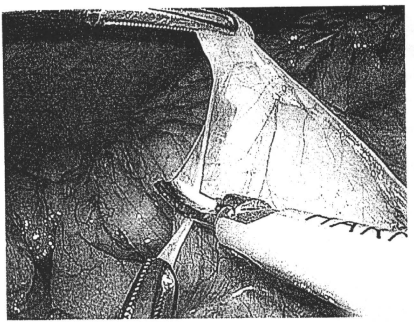

図 2
薄い膜を突破口として十二指腸・膵臓を背側へ落とすようにしながら結腸間膜を授動する.

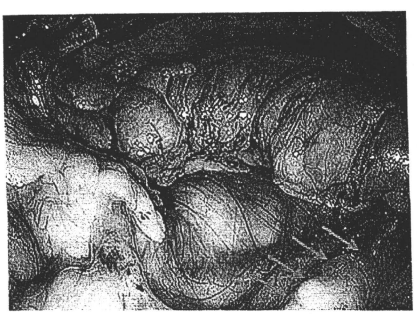

図3 Surgical trunk の膨隆

図 4
Surgical trunk 前面の郭清(D3 リンパ節郭清)が終了.

夫を，以下の3ステージに分けて解説する.

### 1．内側アプローチ変法(modified medial approach)

体位を頭左側低位として大網と横行結腸を頭側へ小腸を左側へ排除して術野を確保すると，結腸間膜に十二指腸前面が透見できる薄い膜を見つけることができる(図1)．その膜を切開して突破口を作り(図2)，十二指腸を背側に落とすように剝離を開始する．すると後腹膜下筋膜のラインを同定することができ，これをメルクマールとして背側に向けて愛護的な剝離を外側・頭側・尾側に進めていく．そうすると結腸間膜は内側からかなり授動することができる[4)-6)]．この操作で注意しているのは，エネルギーデバイス等で剝離する場合は十二指腸を熱損傷しないようにすることである．超音波凝固切開装置であればアクティブブレードが触れないように，スパチュラ型電気メスであればいったん腹腔外に出して冷却してから剝離するようにしている．また，剝離操作を十分に行うことで外側からの操作が非常に容易になる．とくに頭側への剝離を十二指腸下行脚すべてが露出する程度まで剝離しておくと，肝彎曲部の脱転は難易度が高い手技ではなくなる．さらに有利なことは，ここまでの操作を行うとほとんどの症例で回結腸動静脈の走行をはっきりと同定できるとい

うことであり，郭清操作に円滑に移行することができる．

### 2．Surgical trunk 前面のリンパ節郭清

最も難易度が高く重要な操作がSurgical trunk前面のリンパ節郭清である．Surgical trunkは門脈系の血流が豊富な静脈であり損傷した場合は大出血をきたす．CTから得られる情報をもとにSurgical trunkの分枝状況や動脈との関係をあらかじめ把握しておくことも，状況判断するうえで重要な役割を持つ．技術的には回結腸動静脈の走行を確認して本幹の走行を想定し(図3)，腹膜や組織を少しずつ丁寧に剝離していくということに尽きる(図4)．剝離操作が頭側に進むにつれて覗き込むような視野になってくるが，このような時には横行結腸間膜の腹側からアプローチして背側からの剝離と交通させる“横行結腸間膜挟み撃ち法”も有用である[6]．腔鏡下操作にまだ自信がない場合は，郭清操作を目的とした計画的な小開腹創を置いて，直視下に行うのもひとつの手段と考えている[7]．

### 3．肝彎曲部の脱転

最後に外側剝離を回腸末端の腹膜から開始する．回盲部をまわって上行結腸を昇っていくが，肝彎曲部を脱転させるには同方向からのアプローチでは限界がある．そこで横行結腸側から大網の癒着を外して横行結腸間膜を授動すると，比較的薄く透ける肝結腸靭帯を同定することができ，切離することで容易に肝彎曲部の脱転を完了することができる．ポイントは内側からの剝離を十分行うことである．

## II．右側結腸癌に対する新しいアプローチ

### 1．単孔式腹腔鏡下手術

“さらに小さく少ない傷で”というコンセプトのもと単孔式腹腔鏡下手術が注目を集めている．胆嚢摘出術を中心に普及し始めたが，Advanced surgeryである大腸切除術に対しては報告もまだ少ない．当科では右側結腸早期癌に対して導入を行っており，その手術手技を中心に示す．

われわれの手技の特徴はAccess portとしてGelport(Applied Medical，Rancho Santa Margarita，CA，USA)を使用していることである．まず臍縦切開(3 cm)にて開腹してGelportを装着，12 mmトロッカー(15 cm)，12 mmトロッカー(10 cm)，5 mmトロッカー(10 cm)の3トロッカーで行う．トロッカー同士の距離を確保し，角度をつけて挿入することができるため，腹腔外での干渉を少なくし，Triangulationの維持もしやすくなる．つまり，特殊なデバイスは必要なく，通常の腹腔鏡下手術と同じ手順で手術を進めることができる．これがGelportを使用する最大の利点である．これまで7例の早期大腸癌に対して単孔式腹腔鏡下大腸癌手術を行った．現時点で術後早期合併症はなく，早期癌に対しては安全にできると考えている(図5)．

### 2．ロボット支援腹腔鏡下手術

直腸癌に対するロボット支援腹腔鏡下手術の導入を目指し，まず左側結腸癌に対して開始した．ロボット支援腹腔鏡下大腸切除術は2002年に報告[8]されて以来，あらゆる大腸疾患への応用が試みられてきた．しかしながら，ロボットアームの可動域の制限や術中セットアップ変更の困難性から普及が進んでいなかった．最近はセットアップの工夫によりワンステップでの手術が可能となり[9][10]，手術件数が増えてきている．当科では現在4例に施行し，順調に経過している．今後は右側結腸癌に対しても導入を予定している．da Vinci Sサージカルシステムはデバイスの操作性に関して自由度が高く，3次元画像をもとに繊細な操作を行うことができる．Surgical trunk前面の郭清操作のように繊細な操作が必要な場面ではとくに有用ではないかと考えている(図6)．

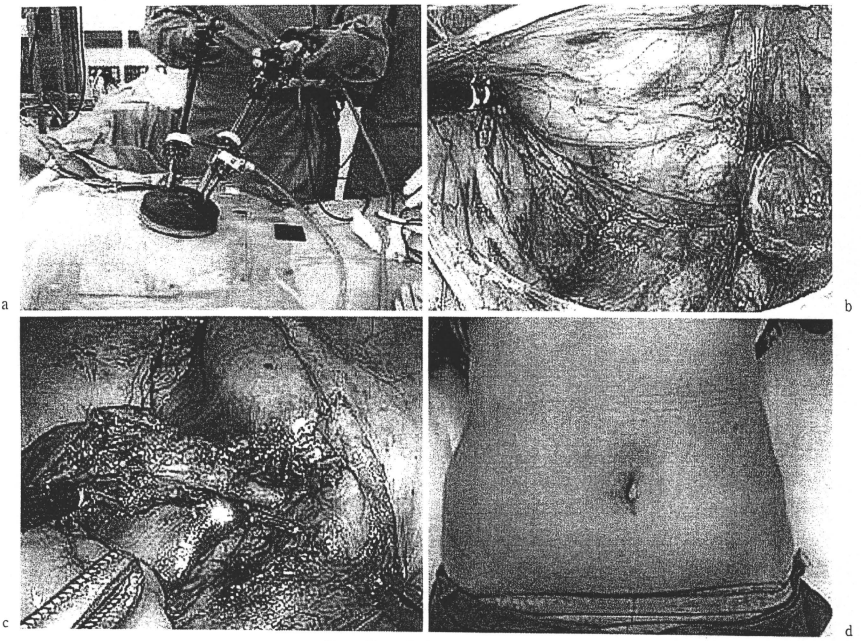


図5 単孔式腹腔鏡下大腸切除術

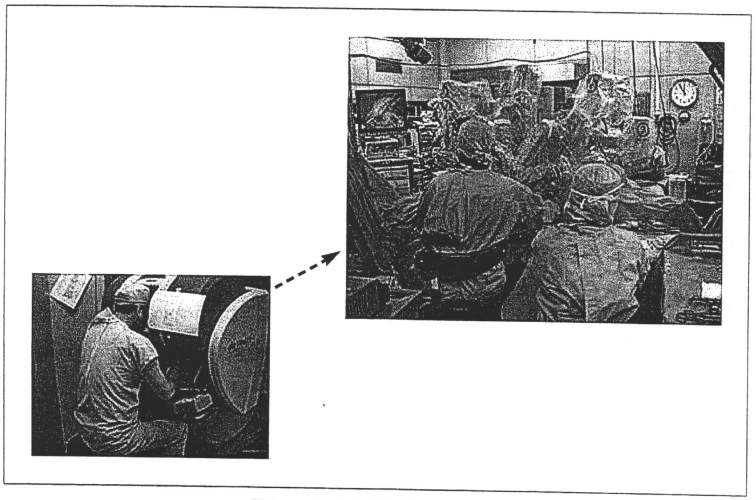

図6 ロボット支援腹腔鏡下大腸切除術

## ま と め

右側結腸癌に対する腹腔鏡下手術を安全に行うための工夫を，**授動操作**と**リンパ節郭清**に注目して述べた．一方，新しいアプローチとして単孔式腹腔鏡下手術やロボット支援腹腔鏡下手術が登場しており，今後注目すべき手法であると考える．

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# A Novel Combined Laparoscopic-Endoscopic Cooperative Approach for Duodenal Lesions

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**Abstract**

***Purpose:*** A surgical approach with minimal invasion and excellent outcome for removal of duodenal lesions, using laparoscopic-endoscopic cooperative surgery (LECS), was established.

***Patients and Methods:*** Two patients underwent the resection of duodenal lesions with our novel LECS approach. Case 1 (age: 49 years; male) had a 20-mm 0–IIa-like lesion (group IV tumor on biopsy) in the duodenal bulb. LECS interventions, performed under general anesthesia, employed a total of four trocars. The extent of lesions was determined with the endoscopic submucosal dissection (ESD) technique. The affected duodenal wall was then perforated before a one fifth turn resection was performed to expose lesions of the whole layer. A tumor, confirmed under laparoscopy, was turned over toward the abdominal cavity to facilitate resection. Case 2 (age: 49 years; female) had 20-mm 0–IIc lesions (group III adenoma) located at the second portion of the duodenum. LECS procedures for duodenal resection were performed in a manner similar to case 1. A total of five trocars were used.

***Results:*** Histologic diagnosis of the tumor in case 1 was tubular adenoma with moderate atypia (size: 20×12 mm). As for case 2, histopathologic findings confirmed a tubular adenoma with moderate atypia (size: 18×18 mm) and an adenoma-negative surgical margin. The postoperative courses, in both cases, were uneventful.

***Conclusions:*** Although only 2 cases were surgically intervened with limited experience, the present novel LECS approach allowed a reliable, adequate resection of tumors located in the duodenum, with abbreviated operation times (156–179 versus 202–229 minutes), minimal bleeding, less postoperative stress imposed on the surgeons, and an uneventful postoperative course, compared to conventional surgical methods.

## Introduction

ENDOSCOPIC SUBMUCOSAL DISSECTION (ESD) of duodenal lesions is practically difficult and is occasionally accompanied by the perforation of eroded luminal walls. Particularly in cases where tumor size and margin are undefined, the reliable, adequate surgical resection of tumors, under laparoscopy, remains a critical issue. Accurate laparoscopic wedge resection of tumors cannot be established as a routine practice because of excessive extent in resected materials. As such, a novel approach, incorporating laparoscopic-endoscopic "rendezvous" procedures, was developed for the reliable, adequate resection of gastric submucosal masses with minimal invasiveness. Although our experience has been limited to only 2 patients, our laparoscopic-endoscopic cooperative surgery (LECS) procedure appears to be feasible and useful for the resection of duodenal lesions.

## Materials and Methods

### *Patients*

Case 1. A 49-year-old male patient was diagnosed as having a duodenal lesion on a regular medical examination in 2006. He had no familial or past medical history of duodenal lesions. The lesion was located in the duodenal bulb, and a follow-up examination, in 2008, showed an enlargement in size. The patient was referred to our hospital. Upon admission, his medical status and biochemical indices did not show any particular abnormalities. Endoscopic studies of the upper digestive tract revealed that there was a 20-mm 0–IIa-like lesion in the front wall of the duodenal bulb. This was

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histologically diagnosed as a group IV adenoma (Fig. 1) that required surgical resection.

Case 2. A 49-year-old female was diagnosed as having a duodenal lesion on a regular medical examination. She had no familial history of duodenal lesions, although she had previously undergone a hysterectomy because of a uterine myoma. A recent endoscopic examination screening identified a 0–IIc lesion at the second portion of the duodenum. Because the lesion was histopathologically diagnosed as a group III adenoma, the patient was immediately referred to our hospital for surgical intervention. A 20-mm 0–IIc lesion was located at a site opposite the duodenal papilla (Fig. 2). Due to the anatomy and tumor staging of the affected site, LECS was adopted as the most practical treatment option.

*Setup for the LECS method*

All LECS interventions were performed under general anesthesia, and the patient was kept supine, with the lower limbs spread laterally apart and supported by a levitator. The performing (i.e., chief) surgeon always stood between the expanded legs, while the assisting surgeon stood on the left side of the patient. The assisting surgeon performed all the laparoscopic procedures. Endoscopic procedures were performed by the endoscopist from the left side of the patient. To facilitate laparoscopic performance, a camera holder (AESOP-9000) was fixed at the right side of the patient (Fig. 3A). A trocar was initially inserted at a site 12 mm to the left-lower abdominal site from the midline/navel, followed by the insertion of four other trocars (Fig. 3B). The jejunum was first clamped with a pair of bulldog clamp forceps under laparoscopy. The duodenum was mobilized from the retroperitoneum.

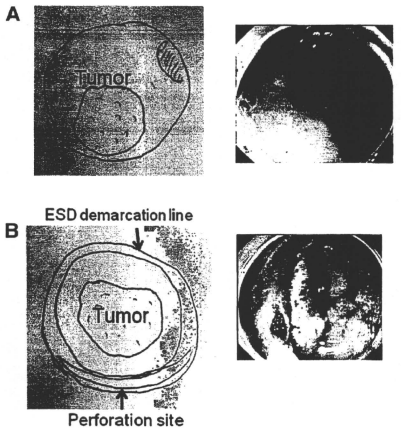


FIG. 1. Endoscopic findings with laparoscopic procedures in case 1. (A) A 20-mm 0–IIa type lesion was located at the duodenal bulb. (B) The whole affected submucosa was dissected, using the ESD technique. A one fifth turn partial perforation under endoscopy was made.

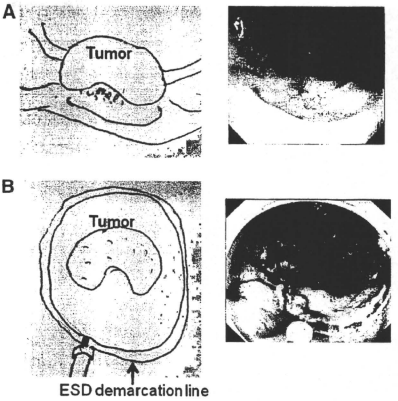


FIG. 2. Endoscopic findings with laparoscopic procedures in Case 2. (A) A 20-mm 0–IIc lesion was located at the second portion of the duodenum. (B) ESD was performed around the tumor with a circumferential resection to facilitate subsequent partial perforation.

*Tumor confirmation and endoscopic procedure*

The right side of the operating table was tilted upward (30 degrees to the horizontal) to facilitate endoscopic observation, and the affected mucosal and submucosal layers around the tumor were circumferentially dissected by using the ESD technique under intraluminal endoscopy. The lesion-sited luminal wall was then artificially perforated by a one fifth to one third turn of the marked line (Figs. 1 and 2).

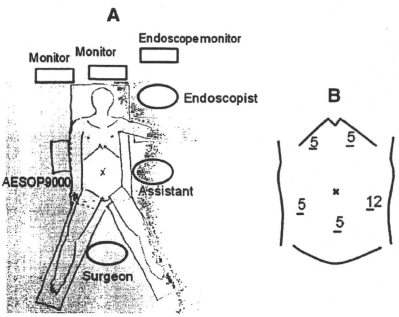


FIG. 3. (A) Setup of the LECS method. (B) Distribution of trocars: A 12-mm trocar was inserted intra-abdominally in a supraumbilical position (12 mm superior to the navel), followed by three or four 5-mm trocars, as indicated.

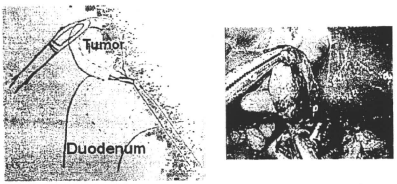


FIG. 4. Surgical procedures involving inversely exposing the lesion intra-abdominally to facilitate tumor resection.

*Laparoscopic dissection and closing the duodenal wall*

The tumor identified under laparoscopy was intra-abdominally inversed, and seromuscular dissection was done. The whole lesion was dissected circumferentially, using an ultrasonically activated device, along the demarcation line (Figs. 4 and 5). The ultrasonically activated device could avoid major bleeding from the resection. The resected tumor was collected in a retrieval bag for further evaluation. The perforated portion of the duodenum was closed by the laparoscopic hand-sewn technique (Fig. 6). A soft tube for drainage was placed near the duodenum, after the operation, for 5 days.

## Results

In case 1, the whole operation lasted 156 minutes, with limited intraoperative blood loss. The resected specimen in case 1 measured 30 mm in length, with a central 20-mm 0–IIa-like mass. Histopathologic studies of the tumor revealed a tubular adenoma with moderate atypia and an adenoma-negative surgical margin. In case 2, the operation time was 179 minutes, and intraoperative blood loss was negligible. The resected specimen measured 30 mm in size, with an 18-mm 0–IIc lesion in the center. Histopathologic studies confirmed that the lesion was a tubular adenoma with moderate atypia, with a size of 18×18 mm, and the surgical margin was also negative.

Before the introduction of LECS, we performed the laparoscopic duodenal resection, using an electrical scalpel and an ultrasonically activated device, in 2 other cases. Operative times were 202 and 229, compared with 156 and 179, minutes in our present 2 cases. If the ranges of operative time were taken as 202–229 and 156–179 minutes in conventional methods and our present LECS method, the operative time was abbreviated by 23–22% with the LECS approach. The longer operative duration in conventional methods was due to visual difficulty in discriminating the resection margin only with the laparoscope and excessive resection of the duodenal wall. In addition, the hand-sewn region induced a large defect in the duodenal wall. Apart from the operative time, an improvement in operative stress imposed on surgeons was also established.

## Discussion

Techniques of focal tumor resection of early-stage and gastric mucosal lesions, using laparoscopy, have previously been described.[1–4] In these surgical procedures, determining adequate resection (i.e., demarcation line) remains a major problem. Excessive resection may cause postoperative transformation of the stomach wall or tissues, while inadequate dissection may result in tumor recurrence (secondary to adenoma-positive margin). Hiki et al. have overwhelmed this shortcoming with their LECS, where intraoperative endoscopy is combined with a manual ESD technique.[5] The resection margin was markedly visible under endoscopy, while the perforation under laparoscopy was executed after the mucosa-submucosa separation to secure a reliable, adequate resection.

In this study, a LECS approach was employed to remove lesions of the duodenum. Despite undefined margins, we achieved preferable focal tumor resection without excessive removal of the surrounding tissues. Further, our minimally invasive resection abbreviated operation time. Previous cases operated on with conventional laparoscopic

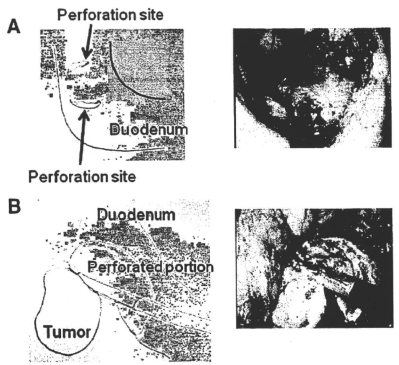


FIG. 5. Intra-abdominal procedures. (A) Partial perforation of the proximal (oral direction) and distal (anal direction) using the ESD technique under endoscopy. (B) the affected whole layer was dissected circumferentially, using an ultrasonically activated device, along the intra-abdominal demarcation line.

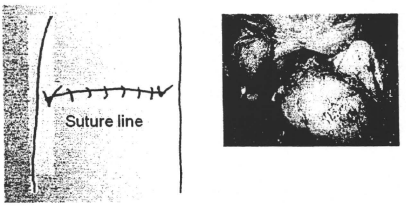


FIG. 6. Intra-abdominal procedures. The incision line was closed by the laparoscopic hand-sewn technique.

wedge resection required 202 and 209 minutes, compared with present cases 1 (156 minutes) and 2 (179 minutes).

A size limit of approximately 3 cm is considered appropriate for duodenal wall resection, because hand-sewing/stitching would be practically difficult in cases where large defects of the wall have been inflicted. Additionally, closures of large defects may also cause postoperative stenosis. Through our limited experience, the current indication of our novel LECS approach for duodenal lesions is limited to adenomas and submucosal tumors. Extreme caution should be exercised for indications other than adenomas and submucosal tumors. In terms of surgical removal adequacy and reliability, the present LECS procedures were comparable to those employed for the surgical removal of gastric submucosal tumors.[6]

This LECS technique was previously performed only with partial gastric resection. Findings on duodenal lesions from PubMed, using keyword inputs such as duodenal local resection, laparoscopy, endoscopy, and cooperative, revealed that studies on cooperative surgery of laparoscopy and ESD technique in combination with the application of endoscopy have not previously been attempted. With reference to the term cooperative, used by Hiki et al.[5] in endoscopic-laparoscopic rendezvous surgery, factors bothering the endoscopists (e.g., prevention of hemorrhages and securing vision field after perforation) are much reduced and buffered by the supportive use of a clamp from the laparoscopic perspective (i.e., from the abdominal side) in our novel approach.

However, intra-abdominally inversing the tumor raises concern about possible intra-abdominal dispersion of the tumor. In a previous study by Ikehara on 90 perforated cases treated by ESD for gastric carcinoma, recurrence was not encountered.[7] Therefore, resection-derived tissue debris, containing small amounts of tumor cells dispersed intra-abdominally, is unlikely to induce tumor recurrence and/or metastasis. As our study was limited to LECS performance on duodenal lesions diagnosed as adenoma, further studies are warranted to confirm our finding on possible intra-abdominal dispersion of tumor-bearing tissues to induce tumor metastasis or recurrence other than adenoma. Further, a novel approach to reduce intra-abdominal tumor exposure has to be innovated to ascertain the nondispersal of tumor debris, which may eventually induce nearby and remote metastases. When we can resolve this issue of intra-abdominal dispersal, the present LECS method can be expanded to cases for the removal of early-stage tumors in the visceral cavity.

## Conclusions

Using the present novel LECS technique on duodenal lesions, it is possible to achieve reliable, adequate resection of tumors located in the duodenum, with operation time abbreviated by 23–22% and less operative stress imposed on performing surgeons, compared with conventional methods. The LECS approach for duodenal tumors would be preferable for resection along a precise cut line, in terms of ensuring negative margins and avoiding unwanted resection, although future studies are required to evaluate the reliability and benefits of LECS in this situation.

## Disclosure Statement

No competing financial interests exist.

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# Genetic Alterations of K-*ras* May Reflect Prognosis in Stage III Colon Cancer Patients Below 60 Years of age

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**Purpose:** Genetic alterations that are closely associated with patient prognosis can be landmarks of definitive therapeutic targets as well as useful biomarkers in human cancer clinics.

**Methods:** Three hundred seventy-eight colorectal cancer (CRC) patients were examined for K-*ras* mutations by single-strand conformation polymorphism (SSCP), with a subsequent 144 young colon cancer (YCC) patients added to validate its prognostic significance.

**Results:** K-*ras* mutations were identified in 161 (43%) of the 378 CRC patients and were significantly associated with tumor location (colon vs. rectum; 80/218 = 37% vs. 81/160 = 51%; $P = 0.0068$) and age (≥60 vs. <60; 103/220 = 47% vs. 58/158 = 37%; $P = 0.049$). The incidence of K-*ras* mutations was 30% in YCC patients as compared to 55% in elderly rectal cancer patients ($P = 0.0004$). K-*ras* mutations significantly correlated with a worse prognosis ($P = 0.0014$) only in 73 curatively resected YCC with stages I–III, but not in other CRCs, which was further validated in the independent set of the corresponding 144 YCC patients ($P = 0.024$). Both univariate and multivariate analyses identified K-*ras* mutations as an independent prognostic factor (HR = 5.5, $P = 0.029$; HR = 3.6, $P = 0.011$) in both learning and validation sets of the curatively resected YCC with stages I–III, respectively, and the prognostic relevance was marked in stage III YCC patients ($P = 0.002$), but not in stages I, II, and IV.

**Conclusion:** In curative YCC, K-*ras* mutations could have excellent prognostic value. Hence, the K-*ras* mutation status could be a good indicator to predict the clinical outcome in curatively resected stage III YCC patients, and K-*ras* pathway inhibition may be a relevant therapeutic target in CRC, excluding YCC patients with no K-*ras* mutation.

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KEY WORDS: **colorectal cancer; k-ras mutation; prognosis**

## INTRODUCTION

Cancer, especially solid tumor, is a dismal disease that can ultimately lead to death. As the optimal strategy for solid tumors, attention has recently been focused on molecular therapies, such as the targeting of c-erbB2/HER2/neu for breast cancer [1,2], c-kit for gastrointestinal stromal tumors (GIST) [3,4], and epidermal growth factor receptor (EGFR) for non-small cell lung carcinoma [5,6]. Genetic alterations of such genes have been occasionally reported to be of prognostic significance [7–10]. As a result, cancer researchers have reached the consensus that the DNA status of therapeutic targets has a prognostic value.

In colorectal cancer (CRC), one of the most frequent causes of cancer-related deaths world-wide, K-*ras* is a critical oncogene with a prevalent mutation. K-*ras* persistently activates diverse onco-pathways, such as Raf/MEK (mitogen-activated protein/extracellular signal-regulated kinase)/ERK (extracellular signal-regulated kinase), PI3K (phosphatidylinositol 3-kinase)/PDK1 (3-phosphoinositide-dependent protein kinase-1)/Akt, and TIAM1 (T-cell lymphoma invasion and metastasis-inducing protein 1)/Rac (a Rho family GTPase) [11]. In CRC, somatic knockout of a mutant K-*ras* gene led to defective tumorigenesis accompanied by reduced expression of vascular endothelial growth factor (VEGF) [12,13], indicating that K-ras pathway activation plays a critical role in tumor progression in CRC.

K-*ras* mutations are an early event in adenoma, a precancerous form of CRC [14], but its prognostic value remains controversial, with both supporters [15–19] and detractors [20–26]. As a result, the American Society of Clinical Oncology (ASCO) 2008 update of recommendations addresses the utility of KRAS gene mutation testing in patients with metastatic colorectal carcinoma to predict response to anti-EGFR

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Additional Supporting Information may be found in the online version of this article.

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monoclonal antibody (MoAb) therapy with cetuximab or panitumumab but did not acknowledge K-*ras* mutation as having any clinical usefulness as a prognostic marker at present [27,28]. We believe that mutations relevant to CRC should be evaluated for their clinical and prognostic significance, not only for predicting outcome but also in the search for a therapeutic target in CRC. In this study, detailed clinicopathological analysis was performed with a larger number of CRC patients than previously evaluated to reach accurate conclusions regarding the clinical significance of K-*ras* mutations.

## MATERIALS AND METHODS

Three hundred seventy-eight patients with CRC were used to identify a subgroup with definite prognosis in terms of K-*ras* mutations and definition of clinicopathological factors.

From among CRC patients surgically resected at Kitasato University East Hospital between 1995 and 2004, 378 cases were investigated. Data on the CRC patients are shown in Supplemental Table I, in which the 6th Japanese Classification of Colorectal Cancer (JCCC), equivalent to the Dukes' stage, was applied.

Patients were divided into two groups, categorized as either elderly, ≥60 years old or young, <60 years old. If 40, 50, 60, and 70 years old were used to define young age, patient numbers below the cut-off were 8 (2.1%), 48 (12.6%), 158 (42.0%), and 281 (74.0%) in 378 CRC patients, respectively (Supplemental Table II). K-ras mutation exhibited the most intense association with age at a cut-off value of 50 (relative ratio = 2.2, $P = 0.02$), followed by 60 (RR 1.5, $P = 0.049$), when significant associations were found, but patients younger than 50 years of age were too few (13% of all CRC patients). We thus used 60 years old as the cut-off. Moreover, CRC was divided into either colon or rectal cancer, with colon cancer further divided into cecal, ascending, transverse, descending, and sigmoid.

According to the JCCC, pT was designated as follows: pT0 (mucosal invasion, M), pT1 (submucosal invasion, SM), pT2 (muscularis propria invasion, MP), pT3 (subserosal invasion or serosal exposure, SS/SE or A1/A2), and pT4 (invasion to the surrounding organs, SI or AI). Factors pN, H, LM, and P represented lymph node metastasis, hepatic metastasis, lung metastasis, and peritoneal dissemination, respectively. pN was defined as pN1/N2, the first/second tiers of lymph node metastasis, respectively. pN1 was defined as the first tier (Pericolic lymph nodes), and pN2 was defined as the second tier (Intermediate lymph nodes). CRC was classified into JCGC stages 0, I, II, III, and IV, based on pT, pN, and pM. Stages 0 and I were equivalent to pT0N0M0 and pT1/T2N0M0, respectively. Stage II was characterized by pT3N0M0. Stage III was defined by the presence of lymph node metastasis without distant metastasis (M0). Finally, stage IV featured distant metastases.

All cases were informative regarding the preoperative values of tumor markers CEA and CA19-9. The cut-off value determined by BRL Laboratory (Tokyo, Japan) was 2.5 ng/ml and 37.0 U/ml, respectively. Patients were followed up for at least 5 years, or until death. Follow-up was at least every 3 months during the first year, and then every 6 months. Assessment included medical history-taking, physical examination, biological tests, determination of serum CEA and CA19-9 levels (evaluated at every visit), colonoscopy, chest radiography, and chest computed tomography (CT; once yearly), abdominal ultrasonography, and abdominal CT (every 6 months). Recurrence was diagnosed on the basis of imaging and, if necessary, either cytological analysis or biopsy findings. Treatment of recurrence or metastasis included surgical resection (if possible), or 5-FU-based chemotherapy or radiotherapy.

All 378 cases were further analyzed for K-*ras* gene mutations and clinicopathological factors, including patient survival. The observation period ranged from 1 to 60 months, with a mean follow-up period of 42.7 months.

### Validation Set for Prognostic Significance of K-*ras* Mutations in 144 Patients With Curatively Resected Young Colon Cancer (YCC) With Stages I–III

An additional and independent set of 144 young colon cancer (YCC) patients, who had undergone curative resection of the tumors with stages I–III at the Kitasato University Main Hospital between 1995 and 2006, was prospectively registered for further validation of the prognostic significance of K-*ras* mutations. They were further analyzed in terms of K-*ras* gene mutations and clinicopathological factors, including patient survival. The 144 patients were observed for 1–60 months, with a mean follow-up period of 42.0 months, and the 5-year disease-specific survival (DSS) rate was calculated.

Adjuvant chemotherapy was recommended largely for curatively resected stage III patients, although it was heterogeneous as standard therapy had not been developed, but administration was carried out for patients who agreed to the anti-cancer drug administration protocols approved by the authors' institution, which were 5-FU-based regimens +/− leucovorin (isovorin) or CPT-11, orally or intravenously. None of the rectal patients in the current study underwent adjuvant radiotherapy either pre- or post-operatively.

The current study was performed in accordance with the clinical research guidelines of the ethics committee of the Kitasato University School of Medicine. All patients gave written informed consent.

### DNA Extraction

After taking fresh samples, surgically resected materials were fixed in 20% buffered formalin for 24–48 hr, routinely processed, embedded in paraffin wax, and cut into 4-μm thick sections. Histological sections were stained with hematoxylin–eosin for histological typing and staging. For simultaneous DNA analysis, the procedures summarized in previous articles were conducted [29–32], as shown below. (1) Sampling of specimens from surgical materials: fresh non-neoplastic colonic mucosa and colorectal/gastric tumors were scraped with disposable bamboo combs (rods made of bamboo with a spatula-like end, 3 mm × 3 mm × 120 mm) to prevent cross-contamination. (2) Extraction of DNA: tissue samples were transferred from the disposable bamboo combs into 400-μl aliquots of lysis buffer, containing 35 mmol/L Tris–HCl (pH 8.8), 175 mmol/L KCL, 300 μg/mL proteinase K, 0.45% Nonidet P-40, and 0.45% Tween 20 (PNT buffer), in 1.5-ml Eppendorf tubes, which were then incubated for 1 hr at 55°C. To inactivate proteinase K, each sample was then incubated for 10 min at 95°C, and 1 ml distilled water was added. After centrifugation (12,000 rpm × 1 min), 5-μl aliquots of supernatant were used for PCR.

### Search for Mutated K-*ras* Genes Using Single-Strand Conformation Polymorphism (SSCP)

Mutations in K-*ras* gene exon 1 (including both codon 12 and 13) and exon 2 (codon 61) were initially screened by non-radioactive single-strand conformation polymorphism (SSCP) analysis [33]: PCR product samples of 10 μl were diluted threefold with gel-loading buffer (95% deionized formamide, 20 mmol/L EDTA, 0.01% bromophenol blue, and 0.01% xylene cyanol) and heated to 95°C for 10 min, followed by quenching on ice. Aliquots of 3 μl were applied to modified polyacrylamide gels [PAFG: 18% polyacrylamide-bis (49:1), 0.5× TBE, 10% glycerol, 10% formamide, 0.05% ammonium persulfate, and 30 μl TEMED] of 120 mm × 150 mm × 0.35 mm. Electrophoresis was performed with 1.5× TBE running buffer at 500 V and 30 mA for 1 hr at room temperature. Detection: Gels were stained using a silver stain plus kit (Bio-Rad, Hercules, CA), with fixation, rinsing, development, and stopping of the reaction. In this

analysis, mutated bands with PCR-SSCP were evident at 1:64 dilution of mutated alleles [30].

### Direct Sequencing

Direct sequencing of 50 DNA samples, 30 with likely mutations and 20 with a likely wild-type, was performed to confirm the K-*ras* mutational status, as previously described [32]. Briefly, amplified DNA was purified from a 4% agarose gel using a QIA Quick Gel Extraction Kit (QIAGEN, Hilden, Germany) and sequenced using a dRhodamine dye terminator cycle sequence kit and 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA).

### Statistical Analysis

Clinicopathological characteristics across CRC groups were analyzed using the $\chi^2$ test, and logistic regression was used for multivariate analysis, with $P < 0.05$ indicating a significant difference. The Kaplan–Meier method was used to estimate cumulative survival rates, and differences in survival rates were assessed using the log-rank test. All patient deaths were cancer-related, and DSS was measured from the date of surgery to the date of death or the last follow-up. On 5-year DSS, patients who survived for more than 60 months were analyzed as survivors.

## RESULTS

A flow chart of our current research, including the learning and validation sets of prognostic relevance in terms of K-*ras* mutation, is shown in Figure 1.

### K-*ras* Mutations Identified in CRC

K-*ras* mutations were identified in 161 of 378 CRC patients (43%) by SSCP analysis (Fig. 2A), consistent with previous reports on CRC [24]. From among the DNA samples examined, 30 CRC cases of presumed K-*ras* mutation and 20 putative cases of no K-*ras* mutation by SSCP analysis were randomly selected to assess the actual mutation using direct sequencing, which confirmed an actual K-*ras* mutation (Fig. 2B). Clinicopathological analysis was performed in the 378 CRC patients to identify basic clinical factors according to the K-*ras* mutational status (Supplemental Table I), which revealed that K-*ras* mutation was significantly associated with tumor location (colon vs. rectum; 80/218 = 37% vs. 81/160 = 51%; $P = 0.0068$), age ($\geq$60 vs. <60; 103/220 = 47% vs. 58/158 = 37%; $P = 0.049$), and histology (degree of differentiation; well/moderate differentiation vs. poor differentiation; 155/353 = 44% vs. 6/25 = 24%; $P = 0.05$). On the other hand, K-*ras* mutation was not associated with parameters such as TNM factors or tumor markers predicting patient prognosis (Supplement Table I). K-ras mutation was found 90.1% in exon 1 (codon 12 or 13) among the 378 cases, and this tendency was preserved in subpopulations such as 90 YCC learning sets (96.3%) and 27 stage III YCC leaning sets (90%).

### Univariate Prognostic Analysis Including K-*ras* Mutational Status in CRC

Univariate prognostic analysis was performed using the log-rank test and revealed that the poor prognosis of CRC patients was significantly associated with pT factor ($P < 0.0001$), pN factor ($P < 0.0001$), histology ($P = 0.019$), H (hepatic metastasis) factor ($P < 0.0001$), LM (lung metastasis) factor ($P < 0.0001$), P (peritoneal dissemination) factor ($P < 0.0001$), vascular invasion ($P < 0.0001$), preoperative serum CEA value ($P < 0.0001$), preoperative serum CA19-9 value ($P < 0.0001$), and operative curability ($P < 0.0001$). Prognostic relevance according to lymphatic invasion could not be assessed using StatView 5.0 software, because there was no excluded case with an absence of lymphatic invasion. The presence of K-*ras* mutations did not have any prognostic significance (Fig. 3A) and therefore more detailed sub-analysis was performed to elucidate the relationship between K-*ras* mutations and clinicopathological factors, including patient prognosis.

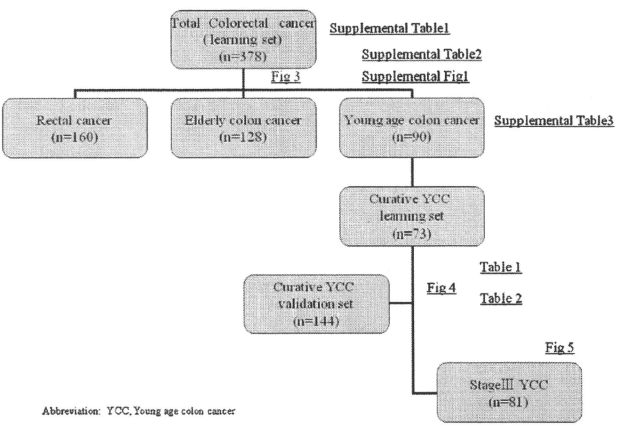


Fig. 1. Flow chart of our analytical process. [Color figure can be viewed in the online issue, available at wileyonlinelibrary.com.]

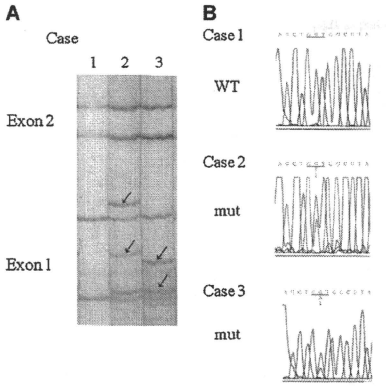


Fig. 2. Detection of K-*ras* mutation in colorectal cancer (CRC) tissues. **A**: Non-RI-SSCP analysis of amplified products of exons 1 and 2 of the K-*ras* gene in CRC. Lane 1, wild-type case; Lane 2, mutant case: Lane 3, mutant case. Arrows indicate mutant alleles. **B**: Direct sequencing of the corresponding cases in Figure 1A. Case 1 shows the wild-type sequence (GGT) of the K-*ras* gene (WT), while cases 2 and 3 have a mutant K-*ras* gene (mut), GTT and GAT, respectively. [Color figure can be viewed in the online issue, available at wileyonlinelibrary.com.]

## K-*ras* Mutation Frequency According to Tumor Location and Age

K-*ras* mutation was significantly associated with tumor location and patient age (Supplemental Table I), suggesting gradual separation of CRC pathogenesis, which could be defined based on these clinical factors. The relationship of K-*ras* mutations with clinical characteristics determined by both location and age revealed that K-*ras* mutations are found significantly less often in YCC (27/90, 30%) than in other CRCs, especially elderly rectal cancer patients (50/89, 55%; $P = 0.0004$).

## Univariate and multivariate Prognostic Analysis, Including K-*ras* Mutations in Curatively Resected YCC With Stages I–III in Both Learning and Validation Sets

The presence of a K-*ras* mutation had a significant predictive value for the 90 YCC patients ($P = 0.0038$; Fig. 3B), while it was not associated with patient prognosis in the other cases of CRC (Fig. 3C,D). Both univariate and multivariate prognostic analysis revealed that K-*ras* mutation was an independent prognostic factor in the 90 YCC cases (Supplemental Table III). Such prognostic relevance was confirmed ($P = 0.0014$), especially in the 73 YCC patients curatively resected with stages I–III (no significant difference in stage IV YCC; Fig. 4A). The presence of a K-*ras* mutation was not associated with any prognostic factors in the 73 YCC (Table I), suggesting that mutated K-*ras* is an independent prognostic factor in curatively resected YCC with stages I–III.

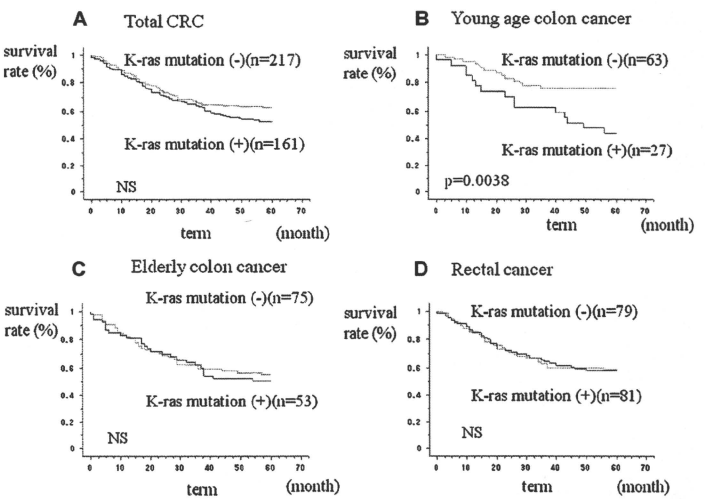


Fig. 3. K-*ras* mutation and prognosis in CRC. **A**: No significant difference in survival between the presence and absence of K-*ras* mutation in 378 CRC cases. **B**: Survival comparison according to K-*ras* mutations revealed a significant difference in young colon cancer patients (YCC; $P = 0.0038$). **C**: No significant difference in survival between the presence and absence of K-*ras* mutation in elderly colon cancer patients, and (**D**) rectal cancer irrespective of age.

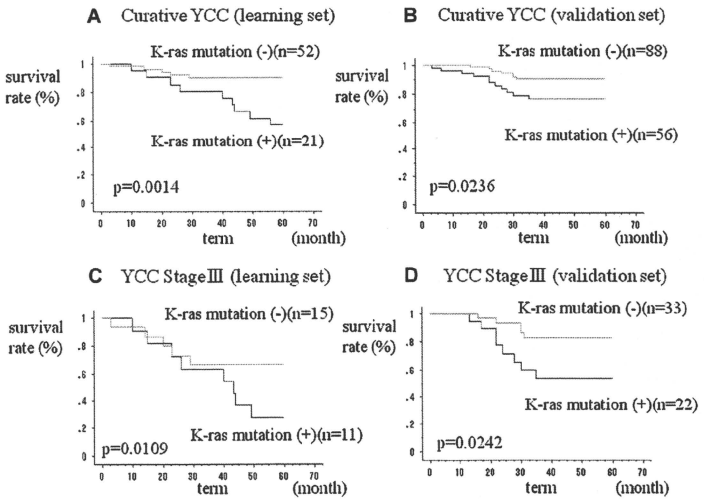


Fig. 4. K-*ras* mutation and prognosis in young colon cancer (YCC). **A**: Significant difference in survival between presence and absence of K-*ras* mutation in 73 curative YCC (learning set; $P = 0.0014$). **B**: Significant difference in survival according to K-*ras* mutation in curative YCC (validation set; $P = 0.0236$). **C**: Significant difference in survival according to K-*ras* mutation in stage III YCC (learning set; $P = 0.0109$). **D**: Significant difference in survival according to K-*ras* mutation in stage III YCC (validation set; $P = 0.0242$).

To confirm these results, an additional 144 cases (validation sets) of curatively resected YCC with stages I–III were newly analyzed as independent cases. The results again confirmed that the presence of a K-*ras* mutation still had significant prognostic value for YCC patients ($P = 0.0236$; Fig. 4B). K-*ras* mutations were not associated with any other parameters predicting outcome (Table I), suggesting that they are not related to carcinoma progression but rather represent definite pathways in YCC. Univariate and multivariate prognostic analyses of the 73 learning sets and 144 validation sets revealed that K-*ras* mutation could be a potent prognostic factor (HR = 5.5; $P = 0.0289$

**TABLE I. K-ras Mutation and Its Clinicopathological and Prognostic Relation YCC**

| | | | K-ras mutational state (%) | | |
|---|---|---|---|---|---|
| | | Number (%) | Mutation (−) (n = 163) | Mutation (+) (n = 87) | *P*-value |
| Learning set (73 curative YCC) | | | | | |
| Sex | M/F | 42 (58)/31 (42) | 32 (76)/20(65) | 10 (24)/11 (35 | NS |
| pT factor | pT0, 1, 2/pT3, 4 | 18 (25)/55 (75) | 15(83)/37(67) | 3 (17)/18 (33) | NS |
| pN factor | Absence/presence | 47 (64)/26 (36) | 37 (79)/15 (58) | 10 (21)/11 (42) | NS (0.057) |
| Histology | Differentiated/poorly differentiated | 69 (95)/4 (5) | 48 (70)/4 (100) | 21 (30)/0 (0) | NS |
| Lymphatic permeation | Absence/presence | 12 (16)/61 (84) | 10 (83)/42 (69) | 2 (17)/19 (31) | NS |
| Vascular permeation | Absence/presence | 12 (16)/61 (84) | 9 (75)/43(70) | 3 (25)/18 (30) | NS |
| Preoperative CEA value | Low/high | 52 (71)/21 (29) | 38 (73)/4 (67) | 14 (27)/7 (33) | NS |
| Preoperative CA19-9 value | Low/high | 65 (89)/8 (11) | 47 (72)/5 (63) | 18 (28)/3 (37) | NS |
| Validation set (144 curative YCC) | | | | | |
| Sex | M/F | 81 (56)/63 (34) | 54 (67)/34 (54) | 27 (33)/29 (46) | NS |
| pT factor | pT0, 1, 2/pT3, 4 | 50 (35)/94 (65) | 28 (56)/60 (64) | 22 (44)/34 (36) | NS |
| pN factor | Absence/presence | 89 (62)/55 (38) | 56 (63)/32 (58) | 33 (37)/23 (42) | NS |
| Histology | Differentiated/poorly differentiated | 141 (98)/3 (2) | 85 (60)/3 (100) | 56 (40)/0 (0) | NS |
| Lymphatic permeation | Absence/presence | 43 (30)/101 (70) | 27 (63)/61 (60) | 16 (37)/40 (40) | NS |
| Vascular permeation | Absence/presence | 47 (33)/97 (67) | 27 (57)/61 (63) | 20 (43)/36 (37) | NS |
| Preoperative CEA value | Low/high | 117 (81)/27 (19) | 74 (63)/14 (52) | 43 (37)/13 (48) | NS |
| Preoperative CA19-9 value | Low/high | 133 (92)/11 (8) | 84 (63)/4 (36) | 49 (37)/7 (64) | NS (0.079) |
| Family history | Absence/presence | 124 (86)/20 (14) | 74 (60)/14 (70) | 50 (40)/6 (30) | NS |

DSS, disease-specific survival; NS, not significant; NA, not assessible.

**TABLE II. Univariate and Multivariate Prognostic Analysis in (A) 73 Curative YCC (Learning Set) and (B) 144 Curative YCC (Validation Set)**

| Variables | | Univariate analysis | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | | DSS (5 years) | *P*-value | HR | 95% CI | *P*-value |
| (A) | | | | | | |
| Histology | Differentiated/poorly differentiated | 83%/50% | 0.0247 | 71.8 | 6.372–810.327 | 0.0005 |
| pN factor | Absence/presence | 98%/50% | <0.0001 | 60.3 | 5.658–643.017 | 0.0007 |
| K-ras mutation | Absence/presence | 90%/57% | 0.0014 | 5.5 | 1.192–25.480 | 0.0289 |
| PreoperativeCA19-9 value | Low/high | 83%/62% | NS | 1.1 | 0.210–6.113 | NS |
| Preoperative CEA value | Low/high | 83%/76% | NS | 0.9 | 0.244–4.036 | NS |
| Sex | M/F | 79%/84% | NS | 0.7 | 0.196–2.646 | NS |
| Vascular permeation | Absence/presence | 92%/79% | NS | 0.4 | 0.041–4.431 | NS |
| pT factor | pT0, 1, 2/pT3, 4 | 100%/75% | NA | | | |
| Lymphatic permeation | Absence/presence | 100%/77% | NA | | | |
| (B) | | | | | | |
| pN factor | Absence/presence | 96%/69% | <0.0001 | 4.3 | 1.090–17.131 | 0.0373 |
| Preoperative CA19-9 value | Low/high | 87%/62% | 0.0041 | 3.9 | 1.119–13.720 | 0.0326 |
| Vascular permeation | Absence/presence | 97%/80% | 0.0144 | 3.7 | 0.453–31.022 | NS |
| K-ras mutation | Absence/presence | 90%/76% | 0.0236 | 3.6 | 1.339–9.948 | 0.0114 |
| Sex | M/F | 84%/87% | NS | 1.3 | 0.559–4.291 | NS |
| Preoperative CEA value | Low/high | 87%/79% | NS | 0.7 | 0.204–2.409 | NS |
| pT factor | pT0, 1, 2/pT3, 4 | 98%/79% | 0.0064 | 0.3 | 0.032–3.370 | NS |
| Family history | Absence/presence | 87%/86% | NS | | | |
| Histology | Differentiated/poorly differentiated | 85%/100% | NA | | | |
| Lymphatic permeation | Absence/presence | 100%/79% | NA | | | |

DSS, disease-specific survival; NS, not significant; NA, not assessible.

and HR = 3.6; $P = 0.0114$, respectively) independently of TNM factors and/or tumor markers, respectively (Table II).

### Curatively Resected Stage III YCC Patients With K-*ras* Mutations Included More Patients With Metachronous Distant Metastasis of CRC

Since K-*ras* mutations were identified as a prognostic factor independent of TNM stage-determining factors, sub-analysis was performed by stage. As a result, K-*ras* mutations had prognostic relevance only in stage III in both learning sets (n = 26, $P = 0.011$, Fig. 4C) and validation sets (n = 55, $P = 0.024$, Fig. 4D). In the 81 stage III YCC patients who were curatively operated (learning plus validation sets), the presence of a K-*ras* mutation had significant predictive value in prognosis ($P = 0.002$; Fig. 5B). Even when stage III YCC patients were subdivided into JCCC N1 and N2 cases, patients with no K-*ras* mutation showed ~80% survival rate (Fig. 5C,D), a result much better than expected for ordinary stage III CRC.

In the 81 stage III YCC cases, K-*ras* mutation was not associated with the administration of adjuvant chemotherapy; 75 patients (93%) underwent 5-FU-based adjuvant chemotherapy (concomitant administration of leucovorin/isovorin, n = 16 or CPT-11, n = 1), orally (n = 59), or intravenously (n = 16). Twenty-nine of the 75 patients had a K-*ras* mutation (39%), while six patients who did not undergo adjuvant chemotherapy included four patients with K-*ras* mutation (67%; no statistical difference), and there was no significant difference in prognosis between the patients with adjuvant chemotherapy and without it (the follow-up periods ranged from 2 to 60 months).

K-*ras* mutations did not have any predictive value in stage 0/I/II/IV patients examined in the current study. Among the 66 stage 0/I YCC patients, only one with a K-*ras* mutation died due to recurrence. Of the 70 stage II YCC patients, 3 died due to recurrence, in which 20 (10%) had a K-*ras* mutation, and 1 of 49 (2%) did not (not statistically significant). In the 19 stage IV YCC patients, K-*ras* mutation was not associated with the survival status (data not shown).

## DISCUSSION

The current study separated YCC patients without a K-*ras* mutation from other CRC patients from a prognostic viewpoint, and found that they showed the best prognosis among all CRC. This finding was unlikely to have resulted from the different distribution of stages within each group that were separated in terms of age and tumor location, because the prognostic relevance of K-*ras* mutation was proven even after adjusting for stage in multivariate analysis (Table II). In particular, stage III YCC patients without K-*ras* mutations clearly showed the best prognosis (~80%) as compared to other stage III CRC patients (50–60%; Figs. 2 and 3). On the other hand, in stage II YCC, a mutated K-*ras* indicated a poorer prognosis (90%) than wild-type K-ras (98%), with very rare recurrence (only 3 patients) among the 69 cases. For stage II YCC patients, we could not find a significant difference in the prognostic value, putatively due to the small number tested and small number of events included, and this should be confirmed in the future. Prognostic markers of stage II CRC, such as DNA ploidy [34], genomic imbalance [35], and microsatellite instability (MSI) [36], have been recognized as vital indicators in patient selection for post-operative adjuvant chemotherapy.

Stage III YCC patients without K-*ras* mutations had a 5-year survival rate of about 80% after surgery, comparable to that of stage II CRC patients [35]. This finding suggested that stage III YCC without a K-*ras* mutation can be recognized as stage II CRC from a prognostic viewpoint, and treated similarly, including adjuvant chemotherapy. For stage III CRC, oxaliplatin-including regimens (FOLFOX or FLOX) were demonstrated to be more effective than surgery alone in the MOSAIC trial [37] and the NSABP C-07 trial [38]; however, an adjuvant effect was achieved in only 6–7% of stage III patients or possibly in high-risk stage II patients [37]. As FOLFOX is expensive and labor-intensive, and also has serious complications, the selection of patients who truly need potent adjuvant chemotherapy is eagerly anticipated. The present study indicates that K-*ras* mutations could be a biomarker for patient selection in stage III CRC. RASCAL-2 is a larger version of RASCAL [39], the largest survey (at that time) of K-*ras* mutations in primary tumor tissues, which included data collected

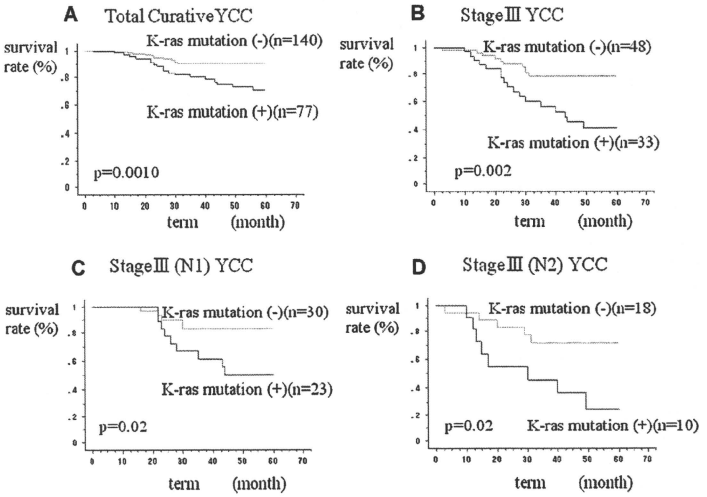


Fig. 5. Prognostic significance of K-*ras* mutation in stage III YCC in curable cases. **A**: Validation of significant difference in survival comparison between presence and absence of K-ras mutation in 217 YCC cases ($P = 0.0010$). **B**: Significant difference in survival according to K-ras mutation in stage III (Dukes C) YCC ($P = 0.002$). **C**: Significant difference in survival according to K-ras mutation in stage IIIA (N1) YCC ($P = 0.02$). Note that stage IIIA (N1) YCC patients without a K-ras mutation had more than an 80% survival rate. **D**: Significant difference in survival according to K-ras mutation in Stage IIIB (N2) YCC ($P = 0.02$). Note that Stage IIIB (N2) YCC patients without K-ras mutation had ~70% survival rate.

by groups from 13 countries on the prognostic importance of K-*ras* mutations. RASCAL-2 examined over 4,000 CRC patients and revealed that K-ras mutations had prognostic significance in stage III CRC [40]. RASCAL-2 may be so huge that K-*ras* mutations would have a prognostic impact even if patients were not limited to YCC; however, our results revealed that K-ras mutations did not have any significant impact on prognosis in CRC other than YCC (data not shown). RASCAL-2 showed that only one mutation on codon 12, glycine to valine, found in 8.6% of all patients, had a statistically significant impact on failure-free survival ($P = 0.004$, HR 1.3) and overall survival ($P = 0.008$, HR 1.29), suggesting that this mutation appeared to have a greater impact on outcome in stage III CRC cancers (failure-free survival, $P = 0.008$, HR 1.5; overall survival $P = 0.02$, HR 1.45) than in stage II tumors (failure-free survival, $P = 0.46$, HR 1.12; overall survival $P = 0.36$, HR 1.15). Our SSCP analysis did not reveal the full profile of each mutation, and we would like to elucidate such associations in the near future.

CRC has been recently proposed to originate in two pathways, MSI and chromosomal instability (CIN) [41]. MSI shows a diploid pattern of DNA content, while CIN has an aneuploid pattern. MSI is more characteristic of proximal colon cancer [42] and young CRC [43], which made us speculate that YCC includes more MSI cases than other CRC. Moreover, a K-*ras* mutation was found in only 13% of MSI CRCs [44], indicating that the mutation is more characteristic of CIN than MSI. Hence, we suppose that YCC without a K-*ras* mutation and with a good prognosis largely reflects MSI, consistent with a report that MSI showed a better prognosis than non-MSI [45]. Nevertheless, CRC sometimes harbors both phenotypes (MSI and CIN), and CIN is the dominant phenotype for aneuploidy [46], which is why K-ras mutation, due to its phenotypic dormancy, clearly showed a poor prognosis in YCC in the current study. We are interested in the relationship of both K-ras mutation and the MSI status with patient prognosis in YCC. On the other hand, even in YCC without a K-ras mutation, several patients had a poor prognosis. This may have been caused by B-*raf* mutation, which has a dismal prognosis in microsatellite-stable CRC [47], and such cases can be included in YCC without K-ras mutation. K-ras mutation might be a marker for MSI and not a prognostic indicator itself. Allowing for these findings, we are planning to profile MSI/B-*raf* mutations in combination with the K-*ras* mutational status in order to clearly explain the prognostic status of YCC in stage III.

We interpreted our results to mean that YCC without a K-*ras* mutation represents patients with a normal K-*ras* pathway. K-*ras* pathway activation may be closely associated with prognosis in CRC, and could be a therapeutic target for most CRC cases (except YCC without K-*ras* mutation). Patients with an abnormal K-*ras* pathway through the activation of either upstream or downstream oncogenes, such as EGFR [48], PI3K [49], and B-raf [50], are similar to those with K-*ras* mutations from a biological viewpoint because the K-*ras* pathway is similarly activated. On the other hand, patients with a normal K-*ras* pathway may show biologically different behavior from those with K-*ras* mutations because the K-*ras* pathway is not activated.

As an optimal strategy for solid tumors, attention has recently focused on molecular therapies by identifying genetic alterations that have been of prognostic value [7–10]. On this basis, the authors suggest the K-*ras* pathway as a therapeutic target for CRC. On the other hand, the K-*ras* mutational status was recently demonstrated to