日臨外会誌　68（1），205—209，2007

症　例

成人前仙骨部 epidermoid cyst の1例

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症例は50歳，男性．平成17年1月頃より便柱の細小化を自覚した．同年9月に近医を受診し，直腸腫瘍疑いにて当科紹介となった．直腸指診では直腸後壁から両側方にかけて表面平滑，弾性硬の腫瘤を触知した．骨盤部CTで前仙骨部に7cm×7cmの嚢胞性病変を認めた．骨盤部MRIでは腫瘍はT1強調像で低信号，T2強調像で高信号であった．前仙骨部のdevelopmental cystを疑い，経仙骨的腫瘍摘出術を施行した．病理組織学的検査では，嚢胞壁は異型に乏しい重層扁平上皮で覆われており，内容物は角化物であった．嚢胞壁には明らかな皮膚付属器の構造を認めず，epidermoid cystと診断された．前仙骨部の嚢胞性腫瘤はdevelopmental cystと定義され，そのうちのepidermoid cystは，扁平上皮癌の発生が報告されており外科的切除する必要があると考えられた．

索引用語：前仙骨部腫瘍，epidermoid cyst，developmental cyst

緒　言

骨盤部において直腸後面と仙骨前面および腹膜翻転部に囲まれた部位は胎生期にcaudal endが存在し，多数の胎児性組織が集合するため，種々の腫瘍が発生する可能性が高い部分とされる[1]．今回われわれは，成人前仙骨部epidermoid cystの1例を経験したので報告する．

症　例

患者：50歳，男性．

主訴：便柱の細小化．

家族歴：特記すべきことなし．

既往歴：特記すべきことなし．

現病歴：平成17年1月頃より便柱の細小化を自覚した．同年9月に近医を受診し，直腸腫瘍を疑われて当科に紹介となった．骨盤部CTおよびMRIにて前仙骨部に腫瘤を認め，精査加療目的にて入院となった．

入院時現症：身長171.5cm，体重70.5kg．直腸指診にて直腸後壁から側方にかけて表面平滑・弾性硬の腫瘤を触知した．疼痛，発熱は認められなかった．腹部は平坦で，腫瘤を触知しなかった．

血液生化学検査：血液一般，生化学，尿検査に異常は認めなかった．腫瘍マーカーはCEA 3.4ng/ml，CA19-9 22.4U/mlと正常範囲内であった．

骨盤部CT検査：前仙骨部に7cm×7cmの嚢胞性病変を認めた．病変は直腸を後方から取り囲むように存在し，被膜様構造を有する嚢胞性病変であり，周囲との境界は明瞭であった（図1）．

骨盤部MRI検査：直腸を後方より取り囲むように単房性の嚢胞性病変が存在し，内部は均一で，T1強調

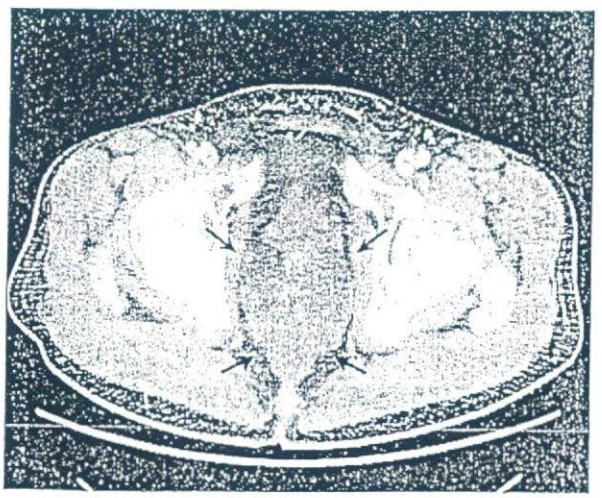

図1　骨盤部CT検査：前仙骨部に7cm×7cmの嚢胞性病変を認めた．

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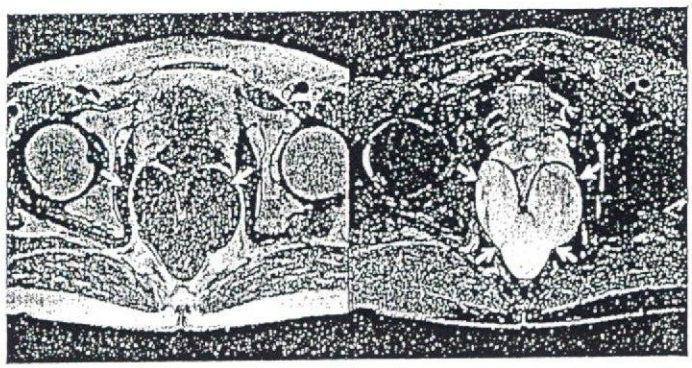


A　　B

図2　骨盤部 MRI 検査：T1強調像で低信号(A)，T2強調像で高信号であった（B）.

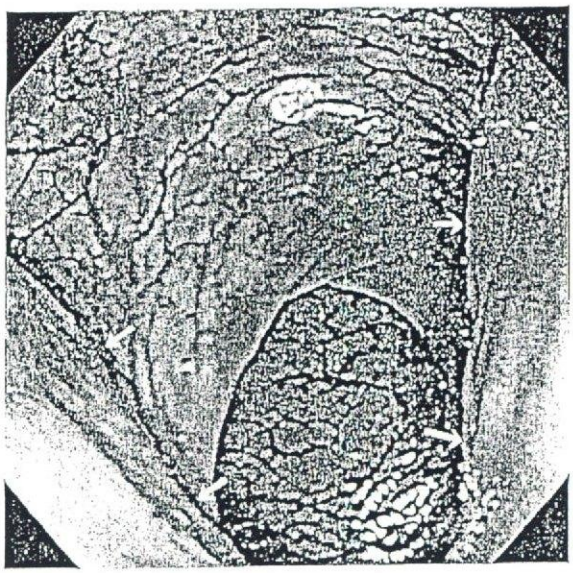


図3　下部消化管内視鏡検査：直腸後壁から側壁にかけて壁外性病変の圧排を認めた.

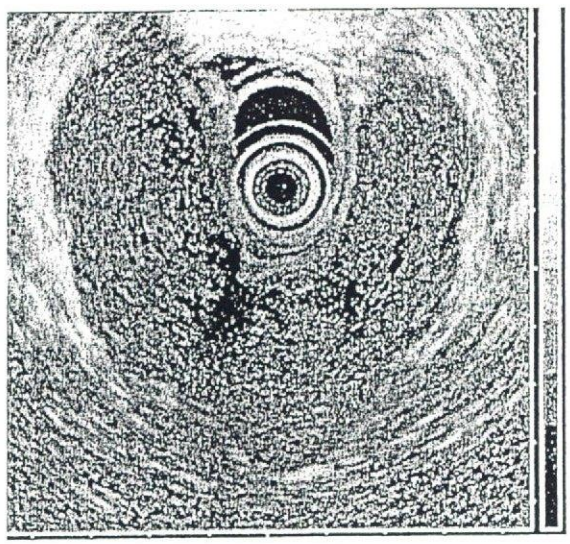


図4　経直腸的超音波内視鏡検査：腫瘍の辺縁は高エコーで，腫瘤内部は不均一な低エコーであった.

像で低信号（図2 A），T2強調像で高信号であった（図2 B）.

下部消化管内視鏡検査：直腸後壁から左右側壁にかけて壁外性病変によると思われる圧排を認めたが，粘膜に異常は認められなかった（図3）.

経直腸的超音波内視鏡検査：腫瘍辺縁は高エコー，腫瘍の内部は不均一な低エコーで，直径は7 cm であった（図4）.

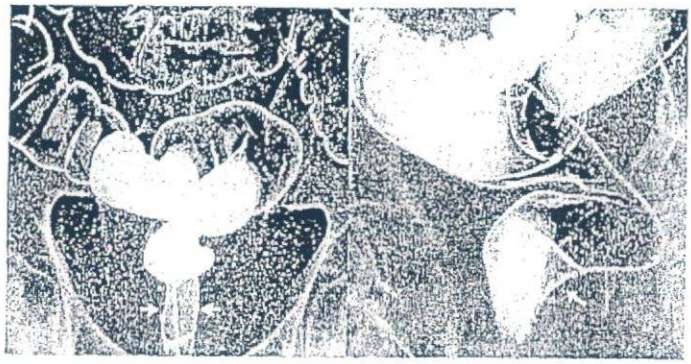
注腸造影検査：腸管壁外から下部直腸への圧排像を認めたが，直腸の壁構造は正常に保たれていた（図5 A，B）.

手術所見：前仙骨部の developmental cyst と診断し，経仙骨的腫瘍摘出術を施行した．尾骨を切除し前仙骨部に入り，腫瘍を確認した．腫瘍は被膜に覆われ，直腸壁や周囲臓器へ明らかな浸潤はなかったが，仙骨に一部炎症による癒着を認めた.

摘出標本肉眼所見：粥状の内容物を含む単房性の嚢胞状病変で，直径は10cm× 8 cm であった（図6）．腫瘍内容物を用いて腫瘍マーカーを測定したところ，CEA 1060.5ng/ml，CA19-9 7245.0U/ml と高値を示した.

病理組織学的検査：嚢胞壁は異型に乏しい重層扁平上皮で覆われており，内容物は角化物であった．嚢胞壁には明らかな皮膚付属器の構造は認められず，epidermoid cyst と診断された（図7）.

術後経過：手術後9カ月が経過したが，再発は認められていない.



A　　B

図 5　注腸造影検査：腸管壁外から下部直腸への圧排像を認めた（A：正面像，B：側面像）．

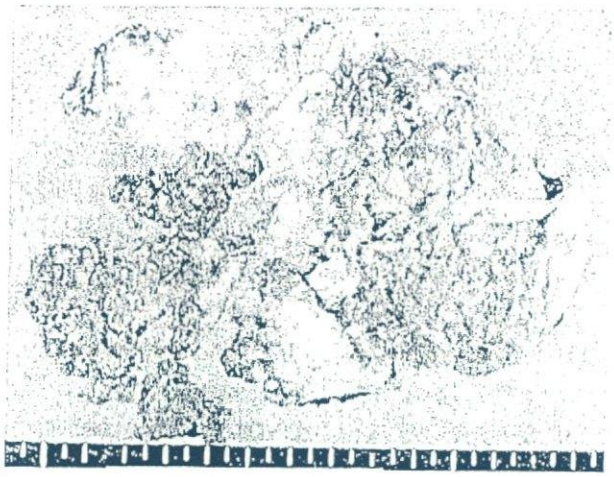


図 6　摘出標本肉眼所見：粥状の内容物を含む単房性の嚢胞状病変で10cm × 8 cm であった（腫瘍割面）．

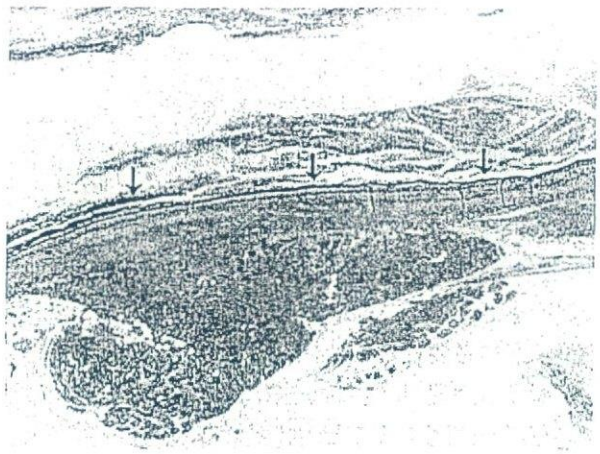


図 7　病理組織学的検査：嚢胞壁は重層扁平上皮で覆われており，内容物は角化物であった（H.E. 染色，×40）．

考　察

前仙骨部は，胎児の発育過程で内胚葉，外胚葉，中胚葉のすべてが関与し，複雑な変化を遂げるため，種々の先天性腫瘤が生じる可能性がある部位である[1]．Hawkins ら[2]はこの前仙骨部に発生した先天性腫瘤のうち嚢胞状腫瘤を developmental cyst と定義し，dermoid cyst, epidermoid cyst, mucus-secreting cyst（tailgut cyst）の 3 種に分類した．本邦での頻度は dermoid cyst 15%，epidermoid cyst 54%，mucus-secreting cyst 31%とされている[3]．前仙骨部 epidermoid cyst の本邦報告例は，医学中央雑誌における1983年から2006年 9 月までの症例報告および会議録を含めた検索では51例であった．

前仙骨部 epidermoid cyst には特異的症状はなく，腫瘍の増大に伴う疼痛や違和感，便秘などが主症状となるが，偶然に発見される症例も多い．

術前診断としては，CT や MRI などの画像診断が用いられているが，術前に dermoid cyst, mucus-secreting cyst，奇形腫などと鑑別診断をすることは困難である[5]．今回検索を行った報告例の中で，術前に穿刺生検が施行され epidermoid cyst と示唆された症例は認められたが[6]，術前に確定診断に至った症例はなかった．穿刺生検は感染や扁平上皮癌合併の場合に播種の可能性があるため，注意が必要と思われる．

今回検索した51例のうち 3 例に扁平上皮癌の合併を認めた[7)~9)]．また，嚢胞内容の腫瘍マーカーを測定した報告例は 3 例であり，いずれも嚢胞内容 CEA の上昇を認めている[8)10)11)]．嚢胞内容 CEA 高値 3 症例中 1 例に扁平上皮癌の合併を認めている．この症例では嚢胞内容の CEA 高値は扁平上皮癌の合併の診断に有用としている[8)]．しかし他の 2 症例および本症例では嚢胞内容の CEA 高値を認めたが腫瘍本体の悪性所見は認められず，腫瘍内容の CEA 高値は必ずしも扁平上皮

癌の合併に結びつくものではないと考えられた．前仙骨部tailgut cystにおいても嚢胞内容のCEAの高値症例の報告を認めたが，扁平上皮癌の合併は認められなかった[12]．CEAは正常組織上皮からも産生されており，嚢胞中のCEAが高値であったのは，嚢胞の上皮細胞から分泌されたCEAが濃縮・蓄積したためと考えられた．

Developmental cystは感染しやすいとされ，感染後は腫瘍の完全切除が困難で再発率も高い[13]．また，先に述べた様に本腫瘍には悪性化の可能性があることより，治療としては外科的に完全切除する必要があるとされている[14]．術式はLocalioら[15]は8cm以下，Stewartら[16]は10cm以下を経仙骨的切除の適応であると報告しており，報告例においても経腹的切除術より経仙骨的切除術が多く施行されている．また近年は腹腔鏡下での摘出症例も報告されている[17]．

治療成績について，developmental cystのほとんどが良性であるが[18]，扁平上皮癌合併の3症例中予後不明の1症例を除いて2症例とも1年前後で再発死亡している．本例を含め完全切除された症例の多くは良好な経過をたどるが，扁平上皮癌の合併例は極めて注意深い観察が必要と考えられる．このため腫瘍の完全切除と術後の病理組織学的検査において扁平上皮癌の合併の有無を確認することが肝要である．

結　語

前仙骨部に発生したepidermoid cystの1例を経験したので報告した．本腫瘍では感染や扁平上皮癌の発生の可能性があることより，外科的切除の必要があると考えられた．

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A CASE OF AN EPIDERMOID CYST IN THE ANTERIOR ASPECT OF THE SACRUM IN AN ADULT

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A 50-year-old man who had noticed an increasing thinning of fecal column since January 2005 visited a hospital in September of the year and was referred to our hospital with a suspicion of a rectal tumor. On digital examination an elastic-hard tumor with smooth surface was palpable in the posterior wall of the rectum extending bilaterally. Pelvic CT scan disclosed a 7×7cm cystic lesion in the anterior aspect of the sacrum. Pelvic MRI of the tumor showed low signal intensity on T1-weighted images and high signal intensity on T2-weighted images. A developmental cyst in the anterior aspect of the sacrum was suggested, and a transsacral tumorectomy was performed. Histopathological studides showed that the cystic wall was covered with stratified squamous epithelium lacking in atypia and the content of the cyst was keratinized material. No apparent structure of appendages of the skin was seen in the cystic wall. Thus epidermoid cyst was diagnosed.

Cystic tumors in the anterior aspect of the sacrum are defined as developmental cysts. Epidermoid cyst of them has been reported to develop squamous cell carcinoma, so that it would require surgical resection.

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ORIGINAL ARTICLE

Trefoil factor 2 in gland mucous cell mucin in the mucous gel covering normal or damaged gastric mucosa using the Mongolian gerbil model

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Abstract

Objective. Trefoil factor 2 (TFF2) is localized in gastric gland mucous cells. The purpose of the study was to determine whether TFF2 and gastric mucin are localized in mucous cells and in the surface mucous gel layer (SMGL) of the normal gastric mucosa or in the mucoid cap adherent to gastric mucosal lesions in Mongolian gerbils. ***Material and methods.*** Gastric mucosal lesions were induced in Mongolian gerbils using oral administration of *Helicobacter pylori* (*H. pylori*), subcutaneous administration of indomethacin, or oral administration of 30% ethanol. Tissue samples were fixed in Carnoy's solution for preservation of the SMGL, dehydrated, and embedded in paraffin. Histochemical staining for gastric mucins and immunostaining for TFF2 were performed. ***Results.*** It was found that surface mucous cell mucin and gland mucous cell mucin were segregated in the SMGL covering the normal gastric mucosa, and the mucin of the mucoid cap covering the mucosal lesions was primarily gland mucous cell mucin. There was a co-localization of TFF2 in gland mucous cell mucin in gland mucous cells, the SMGL, and the mucoid cap. ***Conclusions.*** The co-localization of TFF2 in gland mucous cells and in the adherent mucus suggests a physical interaction between TFF2 and gland mucous cell mucin, and the participation of TFF2 trapped in the adherent mucus functions in mucosal defense, healing, and repair.

Key Words: *Gastric mucin, gastric mucosa, mucoid cap, TFF2, trefoil factor*

Introduction

Two types of mucous cells are present in the gastric mucosa: surface mucous cells covering the foveolae and gland mucous cells located in gastric glands (cardiac mucous cells, mucous neck cells, and pyloric gland cells) [1,2]. We previously reported that these two types of mucous cells secrete different mucins that form the surface mucous gel layer (SMGL) continuously covering the gastric mucosal surface [3], and the gastric SMGL consists of these two types of gastric mucins, surface mucous cell mucin and gland mucous cell mucin, in a laminated structure using paraffin sections from human gastric mucosa fixed with Carnoy's solution [3]. These two types of gastric mucin have different histochemical [1] and biochemical characteristics [4] and may have different functions. The gastric mucosal lesions have a protective cover called the mucoid cap [5–7]. The mucoid cap is a fibrin-based gel with mucus and necrotic cells protecting the epithelial repair process [5].

In addition to mucin, mucous cells secrete the trefoil factor family proteins (TFF). TFF is a family of mucin-associated peptides containing one or two structurally characteristic trefoil domains [8]. In the gastrointestinal mucosa, TFF1 (formerly pS2) is produced primarily by gastric surface mucous cells [9,10]. TFF2 (formerly human spasmolytic peptide (HSP)) is produced by gastric gland mucous cells [9,11], and TFF3 (formerly intestinal trefoil factor

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(ITF)) is found in goblet cells of the small and large intestine [12]. The TFFs are involved in mucosal barrier functions in a cooperative interaction with mucin and in the healing and repair of mucosal damage [8,13–15].

Mongolian gerbils are used to model the pathogenesis of *Helicobacter pylori* (*H. pylori*)-related gastric mucosal lesions and in this model indomethacin- or ethanol-induced gastric mucosal lesions can also be induced [16,17]. Using the *H. pylori*-infected Mongolian gerbil model, we previously reported the increased secretion of gland mucous cell mucins in the mucoid cap covering gastric mucosal lesions in paraffin sections of gastric mucosa fixed in Carnoy's solution. Secretion of TFF2 may increase along with an increase in the secretion of gland mucous cell mucins in these lesions and cooperate with gland mucous cell mucin in the healing and repair of mucosal damage.

Here, we determined the location of TFF2 in the gastric mucous cells, the SMGL in normal gastric mucosa and in the mucoid cap of *H. pylori*, indomethacin- or ethanol-induced gastric mucosal lesions in Mongolian gerbils using tissue sections from stomachs fixed in Carnoy's solution. We demonstrated the co-localization of TFF2 with gland mucous cell mucin both in gland mucous cells and in secreted mucins of the SMGL of normal gastric mucosa and the mucoid cap covering gastric mucosal lesions.

Material and methods

Animals

Seven-week-old specific-pathogen-free male Mongolian gerbils (*Meriones unguiculatus*; Seac Yoshitomi, Fukuoka, Japan) were used. The animals were housed in an air-conditioned biohazard room designed for infectious animals. The animals were divided into four groups consisting of a control normal group (6 animals), *H. pylori*-inoculated group (6 animals), an indomethacin-administered group (6 animals), and an ethanol-administered group (6 animals).

Experimental protocol for H. pylori inoculation

H. pylori, ATCC43504, was obtained from the American Type Culture Collection, Manassas, Va., USA. The strain was grown in Brucella broth (Becton Dickinson, Cockeysville, Md., USA) containing 10% horse serum (v/v) incubated for 40 h at 35°C under microaerobic conditions (15% CO_2) and high humidity with agitation at 150 rpm. A single dose of 1×10^9 colony-forming units (cfu)/ml *H. pylori* in Brucella broth was administered orally using a feeding tube following 24 h of food deprivation (but not water deprivation). Four hours after administration, the animals were allowed free access to water and food. Infected gerbils were killed by cervical dislocation 8 weeks after *H. pylori* inoculation.

Experimental protocol for indomethacin and ethanol administration

Following deprivation of food for 24 h (but not without water), Mongolian gerbils (15 weeks of age) were injected subcutaneously with indomethacin (50 mg/kg in saline). Gerbils of the same age (24 h without food but not without water) were fed orally with 0.8 ml 30% ethanol using a feeding tube. Twelve hours following indomethacin administration or 30 min following ethanol feeding, the gerbils were killed by cervical dislocation.

The optimum time to sacrifice the gerbils in order to examine the mucosal lesions was determined in accordance with previous reports [17–19].

Histopathology and histochemistry

Immediately after collection, the gerbil stomachs were placed in Carnoy's solution for 2 h at 4°C, and then in 100% ethanol at room temperature. Special care was taken not to disturb the SMGL. The stomachs were then sliced along the longitudinal axis into strips. All tissue sections were dehydrated in 100% ethanol, cleared in xylene, and embedded in paraffin. For histological and histochemical examination, serial paraffin sections were cut at 3 μm. The tissue sections were stained with hematoxylin and eosin (H&E) for morphological observation and stained with a dual stain consisting of the galactose oxidase-cold thionine Schiff (GOTS) reaction to demonstrate surface mucous cell mucin and the paradoxical concanavalin A staining (PCS) to demonstrate pyloric gland mucous cell mucin [1]. The tissue sections were also immunostained with anti-TFF2 antibody (mouse monoclonal, 1:20; Novo-Castra, Newcastle upon Tyne, UK) using the indirect immunoperoxidase method (without antigen retrieval). Briefly, the sections were dewaxed and rehydrated, and endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 30 min. The tissue sections were blocked with 5% normal bovine serum albumin in Tris-buffered saline (TBS; 140 mM/l NaCl, 50 mM/l Tris-HCl; pH 7.6) and incubated with the primary antibody at 4°C overnight. After washing in TBS, the slides were incubated with the secondary peroxidase-labeled antibody (DakoCytomation, Glostrup, Denmark) for 60 min. The reaction was developed with

3,3-diaminobenzidine (Sigma Chemical, Poole, UK) containing 0.02% H_2O_2. Sections were lightly counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted in synthetic medium.

As an internal positive control, surface mucous cells were used for GOTS and gland mucous cells were used for PCS and TFF2. Negative controls were obtained by omitting the primary antibody.

This study was approved by and in accordance with the guidelines of the Shinshu University Animal Care and Use Resources Committee.

Results

Macroscopic findings

In *H. pylori*-inoculated animals, the antral mucosa appeared to be thickened, covered by abundant mucus, and showed erosive lesions with hemorrhage primarily in the antrum of the stomach. Intragastric administration of indomethacin or ethanol induced linear hemorrhagic lesions primarily in the corpus of the stomach.

Histology

The gastric mucosa of normal Mongolian gerbils resembles that of other rodents and consists of a forestomach covered by squamous epithelium, and a glandular stomach. The mucosa of the glandular stomach consists of the cardiac, fundic, and pyloric mucosa (Figures 1A, 2A). The SMGL was partially preserved as a thin layer covering the mucosal surface (Figures 1A, B).

In *H. pylori*-inoculated animals, moderate infiltration of inflammatory cells appeared in the lamina propria and the submucosa of the pyloric muocsa. The inflammatory infiltrates consisted of neutrophilic polymorphonuclear cells and mononuclear cells. Inflammation was confined to the pyloric mucosa, where small erosions were frequently observed. Erosive lesions were covered by a mixture of exfoliated cells and eosinophilic materials (the mucoid cap). At the sites of damage, small clusters of surface mucous cells protruding into the lumen were also observed. Erosive sites were covered by immature regenerating epithelial cells.

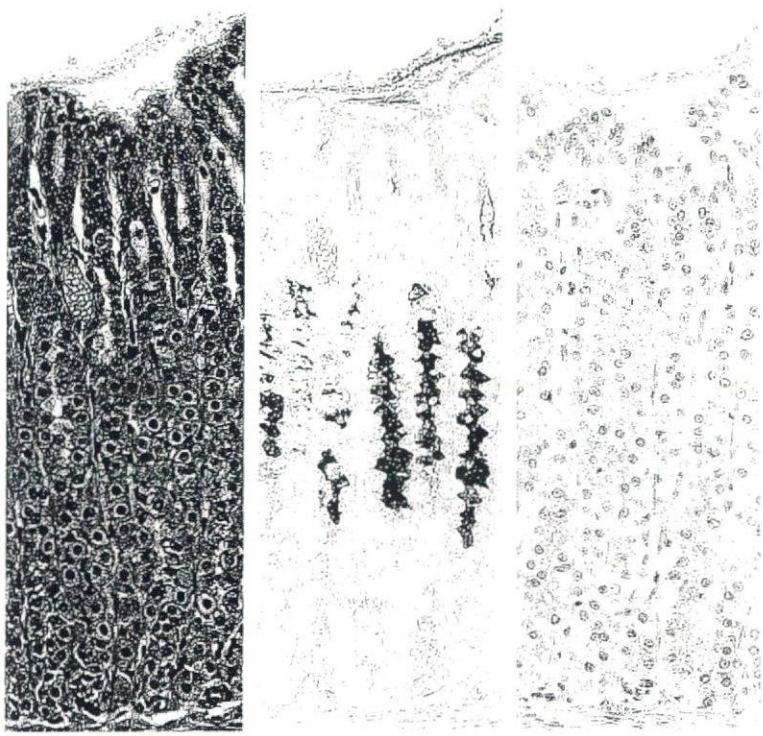

Figure 1. A–C. Histopathology of normal gastric mucosa and gastric mucosa resulting from indomethacin or ethanol treatment. Normal (untreated) fundic mucosa from a Carnoy's solution-fixed, serially sectioned gastric mucosa. A. Typical distribution of surface mucous cells lining gastric foveolae, mucous neck cells, and more basally located parietal and chief cells. The mucosal surface is covered with a thin layer of surface mucous gel layer (SMGL) (H&E, original magnification ×30). B. Surface mucous cells stain blue with galactose oxidase thionine Schiff reaction (GOTS) and mucous neck cells stain brown with paradoxical concanavalin A staining (PCS). In the SMGL, surface mucous cell mucin and gland mucous cell mucin are segregated. (GOTS-PCS, original magnification ×30). C. Trefoil factor 2 (TFF2) is detected within the mucous neck cells. In the SMGL, the distribution of TFF2 is found gland mucous cell mucin reacting with PCS (c.f. Figure 1B) (TFF2 immunostaining, original magnification ×30).

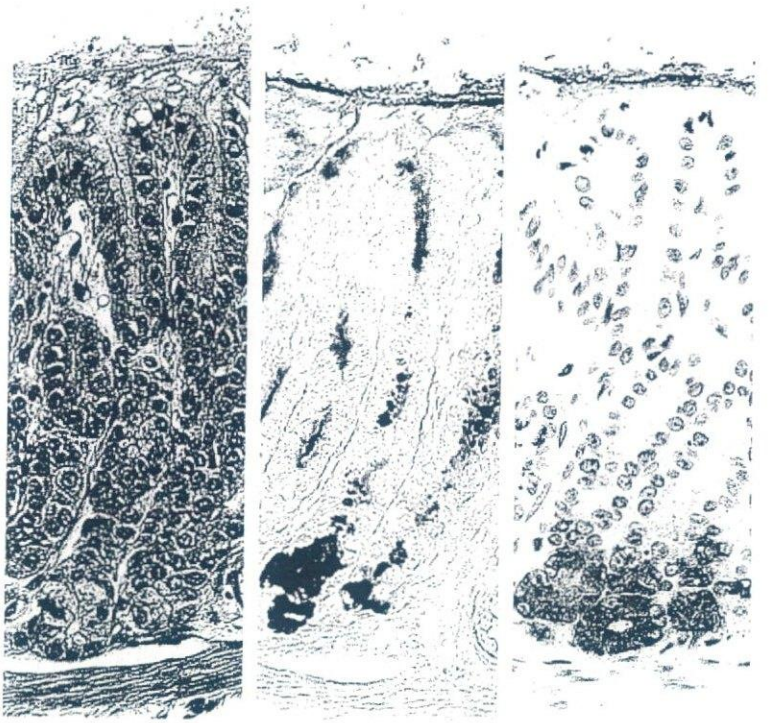

Figure 2. A–C. Normal (untreated) pyloric mucosa from a Carnoy's solution-fixed serially sectioned gastric mucosa. A. Typical distribution of surface mucous cells lining the gastric foveolae and basally located pyloric gland cells. The mucosal surface is covered with a thin layer of the surface mucous gel layer (SMGL) (H&E, original magnification ×40). B. Surface mucous cells stain blue with galactose oxidase thionine Schiff (GOTS) and pyloric gland cells stain brown with paradoxical concanavalin A staining (PCS). In the SMGL, surface mucous cell mucin and gland mucous cell mucin are segregated. (GOTS-PCS procedure, original magnification ×40) C. Trefoil factor 2 (TFF2) is detected within the mucous granules of pyloric gland cells. In the SMGL, the distribution of TFF2 is found with gland mucous cell mucin reactive with PCS (c.f. Figure 2B) (TFF2 immunostaining, original magnification ×40).

The hemorrhagic erosions induced by indomethacin were characterized histologically by various degrees of mucosal damage ranging from luminal superficial damage to deep damage close to the muscularis mucosae. The hemorrhagic erosions induced by ethanol-fed animals were characterized histologically by luminal superficial damage not extending deeper than the glandular neck. The damage was characterized histologically by edema, necrosis, exfoliation of the mucosal epithelial cells into the gastric lumen, and formation of a "mucoid cap" (eosinophilic materials along with cell debris adhered to sites of damage) (Figures 4 and 5). Below the mucoid cap, some elongated epithelial cells were found in the damaged gastric pits (Figures 4 and 5).

Histochemistry

The reactive properties of GOTS-PCS and anti-TFF2 to the gerbils' gastric mucous cells resembled those of the gastric mucous cells of other mammals. Thus, the surface mucous cells stained blue with GOTS and the gland mucous cells stained brown with PCS (Figures 1B, 2B). In the SMGL, surface mucous cell mucin and gland mucous cell mucin were segregated. TFF2 was detected within the mucous granules of gland mucous cells that showing reactivity with PCS (Figures 1C, 2C). In the SMGL, the distribution of TFF2 corresponded to the location of gland mucous cell mucin (Figures 1C, 2C).

In the erosive lesions formed by infecting gerbils with *H. pylori*, secretion of gland mucous cell mucin and TFF2 was markedly increased and was observed as thin strands of mucin originating from the gastric pits, joining the mucoid cap (Figures 3B, C). Gland mucus cell mucin and TFF2 adhered to the sites of damage (Figures 3B, C). After secretion, the distribution of TFF2 corresponded to location of gastric gland mucin. The mucin of the "mucoid cap" observed at sites of mucosal lesions induced by indomethacin or ethanol was primarily found to be PCS-positive gland mucous cell mucin (Figures 4B, 5B). This gland mucous cell mucin adhered to the mucosal sites of damage (Figures 4B, 5B). TFF2 immunostaining was found primarily in PCS-positive gland mucous cell mucin (Figures 4C, 5C).

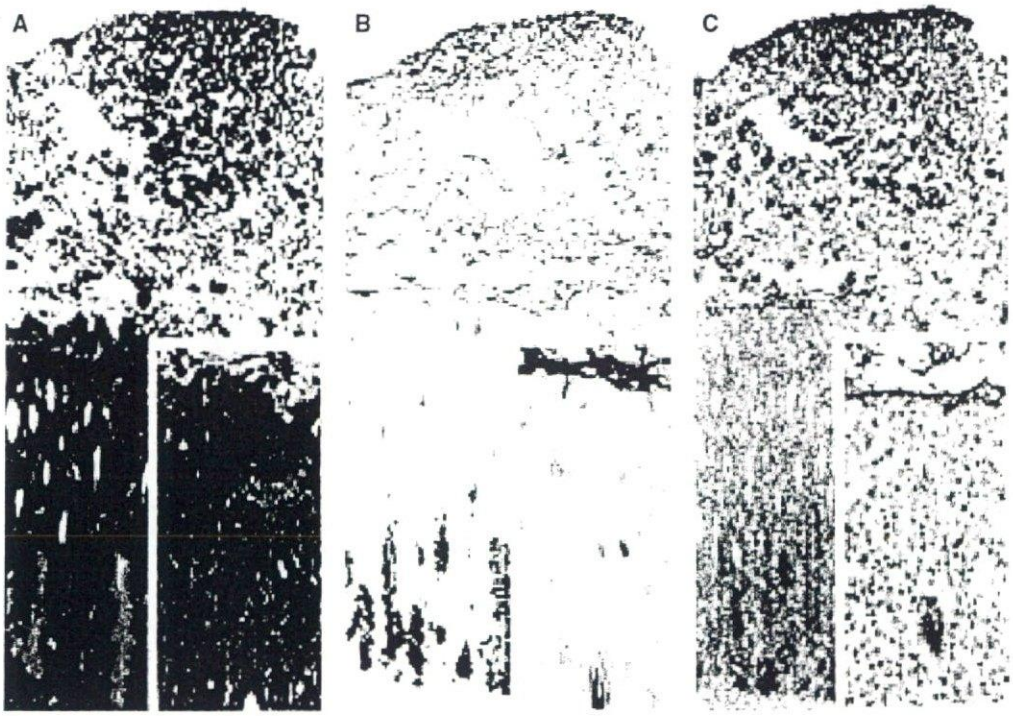

Figure 3. A–C. Pyloric mucosa of *H. pylori*-inoculated Mongolian gerbils from a Carnoy's solution-fixed serially sectioned gastric mucosa. A. Erosion observed: eosinophilic materials along with cell debris adhere to the site of damage. Small clusters of surface mucous cells protrude into the lumen. Inflammatory cell infiltration is present in the lamina propria and in the submucosa (H&E, original magnification ×10 and ×40). B. Gland mucous cell mucin predominates in the mucin of the mucoid cap. Thin strands of mucin from pyloric gland cells are evident and the gland mucous cell mucin joins the mucoid cap. The gland mucous cell mucin adheres to the site of damage (galactose oxidase thionine Schiff-paradoxical concanavalin A staining (GOTS-PCS), original magnification ×10 and ×40). C. Trefoil factor 2 (TFF2) immunostain distribution is found with PCS-positive pylori gland cell mucin (c.f. Figure 3B) (TFF2 immunostaining, original magnification ×10 and ×40).

Discussion

In this report, we examined the immunohistochemical distribution of gastric mucin and TFF2 in Mongolian gerbil gastric mucosa. We showed the co-localization of TFF2 with gland mucous cell mucin in gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells), and in the SMGL covering normal gastric mucosa, and in the mucoid cap over the sites of mucosal damage after secretion from the gland mucous cells.

Previously, we demonstrated how the SMGL of the human normal gastric mucosa could be fixed and preserved in tissue sections using Carnoy's solution. We showed a laminated linear arrangement of the two types of mucin, the surface mucous cell mucin and gland mucous cell mucin, in the SMGL using the GOTS-PCS procedure [1]. We also reported [18–20] that the SMGL of Mongolian gerbil normal gastric mucosa was preserved as thin layers using Carnoy's solution-fixed tissues; and the surface mucous cell mucin and gland mucous cell mucin remained segregated after secretion from gastric mucous cells. This suggests they have different physiochemical properties [4].

Here, we demonstrated the co-localization of TFF2 with gland mucous cell mucin in the SMGL covering the normal gastric mucosa as well as in the gland mucous cells (cardiac gland cells, mucous neck cells, and pyloric gland cells). The presence of TFF2 in the gastric SMGL in Mongolian gerbil normal gastric mucosa concurs with previous results in rats using Western blot analysis, where TFF2 is normally expressed at high levels in the luminal washing and in the mucous gel layer adherent to the antral mucosa [21]. Localization of TFF2 in the gastric SMGL indicates that TFF2 is secreted into the gastric lumen and trapped in the SMGL where TFF2 functions as a structural component of the gastric SMGL acting as a "luminal surveillance peptide" [22] rather than a regulatory peptide. In addition, TFF2 stabilizes the gastric mucus gel [23] leading to a strengthening of the physical barrier function of the SMGL at the luminal surface as shown by Thim et al. [23]. They reported that the addition of TFF2 to mucin solutions resulted in a significant increase in viscosity and elasticity compared with using TFF1 and TFF3. In addition, Tanaka et al., using the rat model, showed that

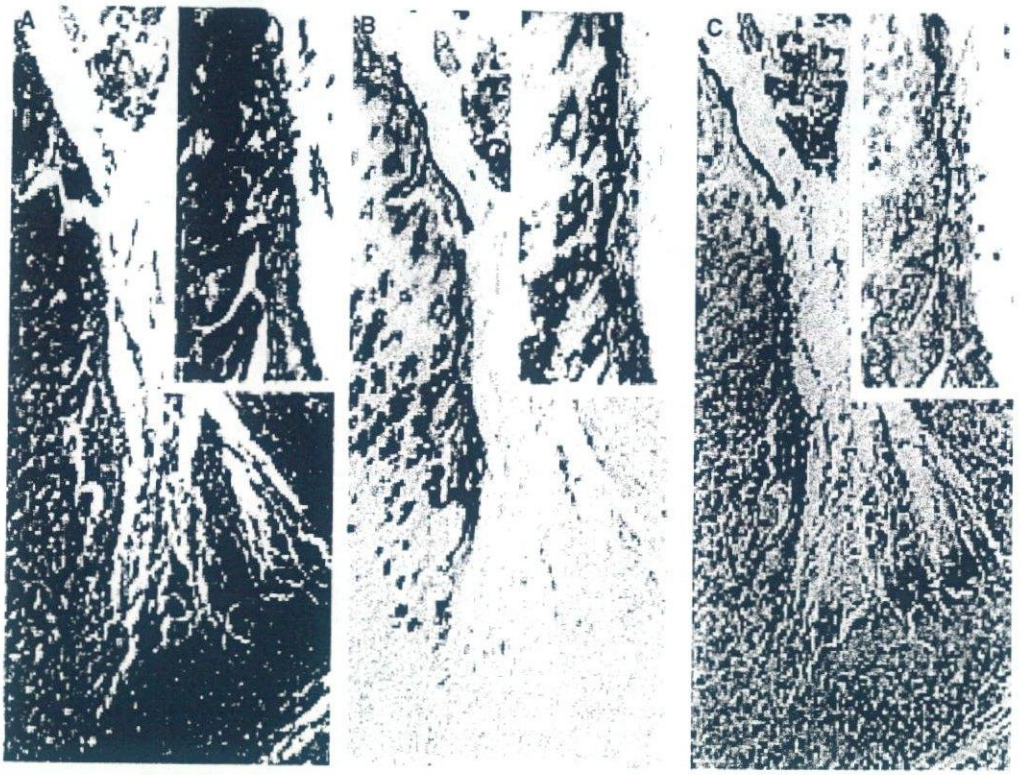

Figure 4. A–C. Fundic mucosa of indomethacin-administered Mongolian gerbils from a Carnoy's solution-fixed serially sectioned gastric mucosa. A. Eosinophilic materials along with cell debris ("mucoid cap") adhere to the site of damage. There was no histological evidence of significant inflammatory cell infiltration into the gastric mucosa (H&E, original magnification ×10, ×40). B. The "mucoid cap" and pyloric gland cells stain positive with paradoxical concanavalin staining (PCS) (galactose oxidase thionine Schiff (GOTS)-PCS, original magnification ×10, ×40). C. Trefoil factor 2 (TFF2) immunostaining distribution corresponds with that of PCS-positive pylori gland cell mucin (c.f. Figure 4B) (TFF2 immunostaining, original magnification ×10 and ×40).

TFF2 interacts with gastric mucin in a manner that inhibits proton permeation through the mucus gel layer [24].

Here, we found orally administered *H. pylori*, subcutaneously administered indomethacin, or orally administered ethanol-induced macroscopic and histological gastric mucosal lesions in the Mongolian gerbil gastric mucosa [17]. In the *H. pylori*-related mucosal lesions, secretion of gland mucous cell mucin was increased and the secreted gland mucous cell mucin adhered to the erosive lesions. Chemically induced mucosal lesions were covered by a mucoid cap, a fibrin-based gel consisting of mucus, and necrotic cells where the underlying epithelial repair process was being protected [5]. Mucin in the mucoid cap was predominantly gland mucous cell mucin. This further suggests different physicochemical properties of the two types of mucin. Gland mucous cell mucin that adheres to the site of mucosal injuries shows that gland mucous cell mucin may be more viscous than surface mucous cell mucin. Secreted TFF2 is trapped in the mucoid cap together with gland mucous cell mucin and adheres to the sites of mucosal damage. Secreted TFF2 may play a role in the healing and repair of gastric mucosal lesions because TFF2 is reported to promote epithelial restitution [22,25] and the report shows that oral administration of TFF2 before injury protects against both indomethacin- and ethanol-induced gastric injury in rats [25].

We previously reported increased secretion of gland mucous cell mucin in *H. pylori*-related chronic active gastritis in both gerbils [18] and humans [26]. Here we showed increased secretion of TFF2 together with gland mucous cell mucin in *H. pylori*-related chronic active gastritis. Increased secretion of TFF2 together with gland mucous cell mucin shows that TFF2 and gland mucous cell mucin may operate synergistically to heal and repair the gastric mucosal lesions.

We found that TFF2 and gland mucous cell mucin co-localized in the gland mucous cells, in the SMGL, and in the mucoid cap over sites of damage, suggesting a physical interaction between TFF2 and gland mucous cell mucin. Our results concur with those of other investigators in demonstrating that TFF2 and MUC6 (the mucin core protein of the gland mucous cell mucin) are co-

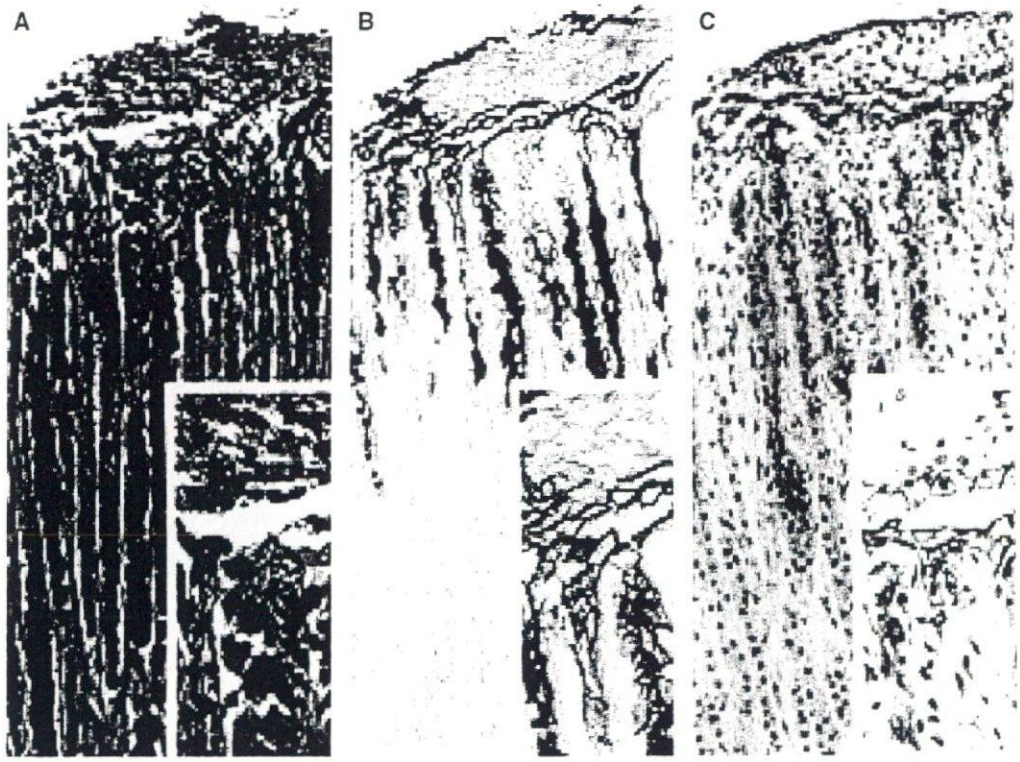

Figure 5. A–C. Fundic mucosa of ethanol-administered Mongolian gerbils from a Carnoy's solution-fixed serially sectioned gastric mucosa. A. Typical lesion showing erosion of the mucosa with predominant loss of the foveolae and loss of the integrity of the apical region of the glands. Eosinophilic materials along with cell debris ("mucoid cap") adhere to the site of damage. There is no histological evidence of significant inflammatory cell infiltration into the gastric mucosa (H&E, original magnification ×10 and ×40). B. The "mucoid cap" and pyloric gland cells stain positive with paradoxical concanavalin staining (PCS) (galactose oxidase thionine Schiff (GOTS)-PCS, original magnification ×10 and ×40). C. Trefoil factor 2 (TFF2) immunostaining distribution is found with PCS-positive pyloric gland cell mucin (c.f. Figure 5B) (TFF2 immunostaining, original magnification ×10 and ×40).

localized in the pyloric gland mucous cells [27]. Similar results were reported for TFF1 and MUC5AC (the mucin core protein of the surface mucous cell mucin) that co-localized in the surface mucous cells in the human gastric mucosa [10,28]. TFF1 was shown to interact with the von Willenbrand factor C cysteine-rich domains that are present within MUC5AC in the mouse [29]. TFF2 may directly interact with MUC6 in a manner similar to the interaction between TFF1 and MUC5AC.

In summary, we demonstrated the participation of TFF2 together with gland mucous cell mucins in the SMGL of normal gastric mucosa and in the mucoid cap over the sites of damage in tissue sections. The secreted TFF2 may participate in mucosal defense, healing, and repair.

Acknowledgements

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Original Paper

Results of 404 Hepatic Resections Including 80 Repeat Hepatectomies for Hepatocellular Carcinoma

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KEY WORDS:
Liver resection; Hepatocellular carcinoma; Repeat hepatectomy

ABBREVIATIONS:
Hepatocellular Carcinoma (HCC); Indocyanine Green Retention Rate at 15 minutes (ICG-R); Blood Transfusion (BTF); Ultrasonography (US); α-Fetoprotein (AFP); des-gamma-Carboxy Prothrombin (DCP); Computed Tomography (CT); Hepatitis B Antigen (HBsAg); Hepatitis C Antibody (HCVAb)

ABSTRACT

Background/Aims: To evaluate our treatment protocol applied to patients with hepatocellular carcinoma. The protocol consists of the selection criteria for hepatectomy, the use of techniques that minimize intraoperative blood loss, strict follow-up after surgery, and an aggressive surgical approach for intrahepatic recurrence.

Methodology: We conducted a retrospective cohort study that included 337 patients with hepatocellular carcinoma treated between 1990 and 2001. The type of resection was selected according to the serum bilirubin value and the indocyanine green retention rate at 15 minutes. Perioperative data and long-term outcome were examined.

Results: We performed 324 initial hepatectomies with an in-hospital mortality rate close to zero. There was one operative death and one hospital death (0.3% each), and the 5-year survival rate for all patients was 53.2%. Eighty repeat liver resections, including 18 third and two fourth, were performed with no mortality, and the 5-year survival rate was 52.9% after the second hepatic resection. The resectability rate for second and third hepatectomies reached 29% and 33% of all patients with isolated liver recurrence, respectively.

Conclusions: Liver resection is a safe and effective treatment modality for hepatocellular carcinoma. Our results are likely attributable to the routine application of our treatment protocol.

INTRODUCTION

With advances in surgical techniques and perioperative care, there has recently been a great improvement in the results of liver resection. For hepatocellular carcinoma (HCC), one of the most common malignancies worldwide, perioperative mortality rates of around 5% are frequently reported (1-3), and many liver surgeons seem to consider these rates acceptable. However, recent studies from specialized medical centers have reported zero mortality rates (4,5). It is therefore important that we re-evaluate the safety of liver resection in patients with HCC.

Hepatic resection has long been held to be the only potentially curative option for HCC. The long-term outcome of 'curative' resection is, however, far from satisfactory because of the high incidence of intrahepatic recurrence (6,7). To improve the surgical outcome of HCC patients, we need to establish effective treatment strategies for such recurrences.

During the last 11 years we have routinely applied our own treatment protocol to HCC patients, which consists of a set of selection criteria for hepatectomy (8,9), combined with surgical techniques aimed at minimizing intraoperative blood loss (9-11), careful follow-up, and an aggressive surgical approach for recurrence (12,13). Using this protocol, we have performed 324 initial and 80 repeat hepatectomies, including 18 third and two fourth resections. Here, we review our 11-year experience of liver resection for HCC and clarify the safety and potential benefits of hepatectomy.

METHODOLOGY

Between January 1990 and December 2001, we have performed 404 potentially curative hepatectomies: 324 initial and 80 repeat liver resections, on 337 HCC patients at the First Department of Surgery, Shinshu University. Potentially curative resection means removal of all gross tumors. These 337 patients, including 13 patients who underwent their initial (8 patients) or second hepatectomy (5 patients) at other institutions, are the subjects of this report. There were 258 men and 79 women, and their mean age was 64 years (range, 21- 85 years). The type of liver resection was defined according to the scheme shown in **Figure 1** (8). If ascites could not be controlled with diuretics preoperatively, liver resection was not indicated. The serum bilirubin value and the indocyanine green retention rate at 15 minutes (ICG-R) were the major parameters for determining the extent of resection. The details of the surgical tech-

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niques and perioperative management have been described in previous reports (8,9). Briefly, liver resection was performed by the forceps fracture method during Pringle's maneuver or the hemihepatic vascular occlusion technique (10). Intraoperative ultrasonography was performed in all cases to assess occult tumors that were not detected by preoperative imaging modalities and to confirm the spatial relationships between the tumors and vascular structures (11). Bleeding from the raw surface of the transected liver was controlled by meticulous placement of surface ligatures. Perioperative blood transfusion (BTF) was defined as the transfusion of whole blood and/or packed red cells either during the operation or within one week afterwards. BTF was performed when the hematocrit level became less than 30% during surgery and less than 20% after. Fresh frozen plasma was transfused to replace 30-40% of the intraoperative blood loss (14). The following types of resection were classified as anatomical: hemihepatectomy, sectionectomy, and segmentectomy. These are defined as resection of a hemiliver, a section, and a segment of the liver, respectively (15). Limited resection and tumor enucleation were considered to be types of "non-anatomical resection". After discharge, patients were closely followed up at our outpatient clinic. All patients underwent routine examinations that included ultrasonography (US) and measurement of α-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) levels for recurrence every 3 months. Computed tomography (CT) with contrast medium was performed every 6 months. The indications for repeat hepatectomy of recurrent HCC were basically decided in a manner similar to those used for the first liver resection, although anatomical resection was rarely indicated. Operative death, death within 30 days of surgery, and hospital death were considered operative mortality. Surgery-related complications included pleural effusion requiring tap, biliary fistula, subphrenic abscess, postoperative hemorrhage from the cut surface of the liver, and wound infection.

Variables Analyzed

The following factors, classified as host-, cancer-, or surgery-related, were analyzed in relation to the disease-free survival after initial hepatectomy. The host-related factors were: age, gender, hepatitis B antigen (HBsAg), hepatitis C antibody (HCVAb), and hepatic functional reserve (ICG-R). The cancer-related factors included number of tumors, maximum tumor diameter, vascular invasion, intrahepatic metastasis, invasion of a tumor capsule, tumor cell differentiation, serum AFP level, and plasma DCP level. The surgery-related factors were: type of resection (anatomical or non-anatomical), margin of tumor clearance (exposed or non-exposed), amount of bleeding (more than 1000mL *versus* less), and perioperative BTF.

Statistical Analysis

Overall survival and recurrence-free survival curves were constructed by the Kaplan-Meier method, and univariate analysis was carried out using the log-rank test. Multivariate regression analysis was performed using the Cox proportional hazard model. Variables to be entered into the regression analysis were chosen on the basis of the results of univariate analyses. Significance was defined as a P value of <0.05. All statistical analyses were performed using StatView 5.0J software (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Clinicopathological Data for 324 Initial Hepatectomies

Seventy-three patients (23%) were positive for HBsAg alone, 211 (65%) for HCVAb alone, 38 (12%)

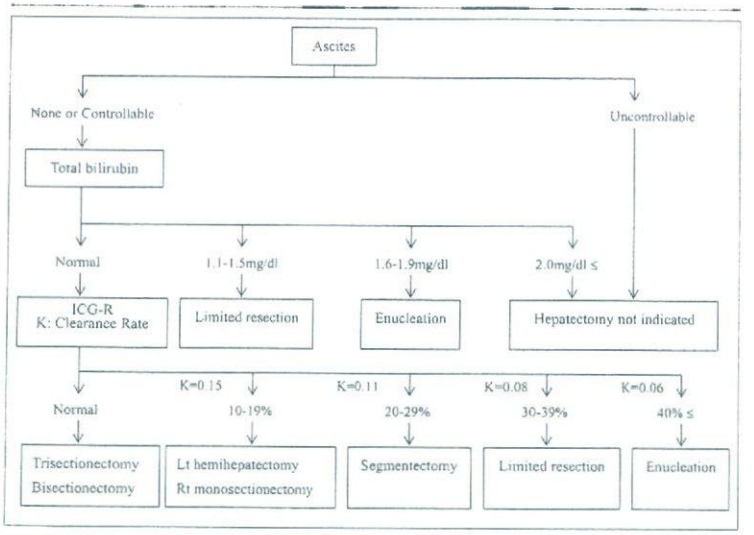

FIGURE 1 Criteria for liver resection in patients with hepatocellular carcinoma. [Reprinted with permission of the publisher (8)]

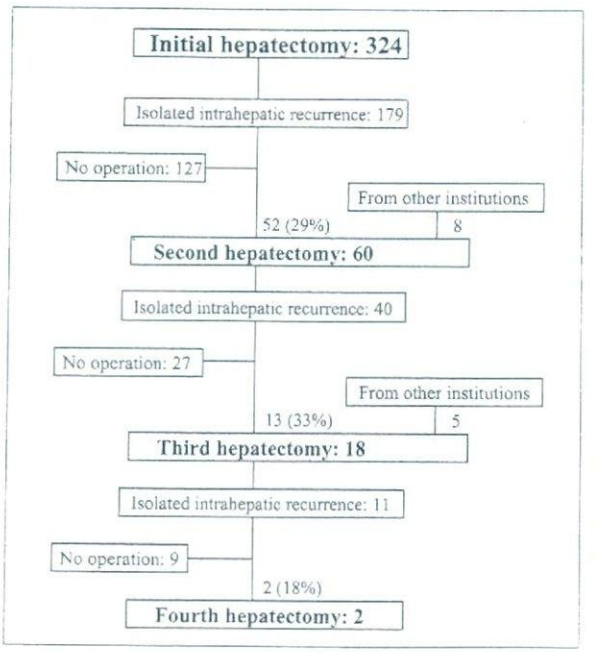

FIGURE 2 Four hundred and four hepatic resections for patients with hepatocellular carcinoma.

were negative for both, and 2 were positive for both. Of the 324 patients, 233 had a solitary tumor and 91 had multiple hepatic lesions, with 27 having three or more lesions. The mean maximum diameter of the tumor was 4.1 (range 0.5- 27.0) cm. Chronic liver disease was demonstrated histologically in 312 patients (96%), including 152 (47%) with cirrhosis and 160 (49%) with chronic hepatitis.

TABLE 1. Univariate Analysis of Possible Prognostic Factors for Cumulative Disease-free Survival in 324 Patients with Hepatocellular Carcinoma Undergoing Initial Liver Resection

Variable		No. of patients	Cumulative disease-free survival (%) at 5 years	p^*
Patient				
Age	>65	195	19.5	NS
	≤65	168	23.6	
Sex	Male	240	21.1	NS
	Female	73	24.1	
HBsAg	Positive	74	38.4	0.0046
	Negative	235	17.3	
HCVAb	Positive	204	15.6	0.0007
	Negative	108	35.0	
ICG-R	>20	132	14.4	0.018
	≤20	179	28.1	
Tumor Number	Single	224	26.8	<0.0001
	Multiple	89	6.8	
Size	>5cm	60	14.7	<0.0001
	≤5cm	253	23.7	
Vascular invasion	Positive	34	7.5	0.0006
	Negative	279	23.8	
Intrahepatic metastasis	Positive	58	0	<0.0001
	Negative	253	26.5	
Capsular infiltration	Positive	195	24.3	NS
	Negative	96	15.5	
Edmondson-Steiner	I-II	234	20.5	NS
	III-IV	69	24.5	
AFP (ng/mL)	>20	187	17.7	0.0018
	≤20	124	27.4	
DCP (AU)	>0.1	117	11.0	<0.0001
	≤0.1	183	28.8	
Surgery				
Type of resection	Anatomic	129	29.4	NS
	Non-anatomic	184	16.3	
Margin of tumor	Exposed	60	17.3	0.014
	Non-exposed	253	23.0	
Amount of bleeding	>1000mL	94	18.9	NS
	≤1000mL	213	23.9	
Perioperative BTF	Yes	31	6.6	<0.0001
	No	276	24.2	

*NS: not significantly different; ICG-R: indocyanine green retention rate at 15 minutes; DCP: des-gamma-carboxy prothrombin; BTF: blood transfusion.

TABLE 2. Results of Cox Multivariate Regression Analysis

Variable	Parameter estimate	Wald X^2	p^*	Hazard ratio†
Perioperative BTF	0.60	7.21	0.007	1.83 (1.18-2.84)
Multiple tumors	0.43	5.86	0.016	1.54 (1.09-2.17)
Presence of intrahepatic metastasis	0.57	8.51	0.004	1.76 (1.20-2.58)
DCP >0.1 AU	0.57	12.95	<0.001	1.76 (1.29-2.40)
HCVAb positive	0.45	7.45	0.006	1.57 (1.14-2.18)

* $P<0.05$ was set as the cut-off for variable elimination; †Values in parenthesis are 95 percent confidence intervals. BTF: blood transfusion; DCP: des-gamma-carboxy prothrombin.

Outcome of Initial Hepatic Resections

Perioperative results: Anatomical resections were performed in 132 patients (41%) and consisted of 1 left trisectionectomy, 20 hemihepatectomies, 41 sectionectomies, and 70 segmentectomies. The remaining 192 patients had undergone limited resection or enucleation of the tumor. The mean and median blood loss of all patients who underwent hepatectomy was 863mL and 650mL, respectively (range, 19-5700mL). Only 32 patients (9.9%) required BTF perioperatively and the mean and median transfusion volumes were 83mL and 0mL (range, 0-3600mL). Surgery-related complications occurred in 107 patients (33%). There was one operative death (0.3%) and one hospital death (0.3%). Both of these patients were included in the analysis of surgical outcome.

Long-term outcome of initial hepatectomy: The mean and median follow-up periods were 44 months and 36 months, respectively (range, 0.4-137). The 1-, 3-, and 5-year survival rates for all patients were 93.7%, 72.4%, and 53.2%, and the disease-free survival rates at 1, 3, and 5 years were 66.9%, 34.8%, and 21.8%, respectively **(Figure 3)**. The following 11 clinicopathological parameters were found to be related to poorer disease-free survival **(Table 1)**: HBsAg-negativity, HCVAb-positivity, ICG-R level above 20%, multiple tumors, maximal tumor diameter more than 5cm, presence of vascular invasion, presence of intrahepatic metastasis, serum AFP level above 20ng/mL, plasma DCP level above 0.1 arbitary unit (AU), tumor-exposed surgical margin, perioperative BTF. Stepwise multivariate analysis by Cox proportional hazard analysis using these 10 variables indicated that perioperative BTF, presence of multiple tumors and intrahepatic metastasis, plasma DCP level above 0.1 AU, and HCVAb-positivity were independent significant risk factors contributing to disease recurrence **(Table 2)**.

Tumor recurrence after first liver resection: Among the 324 patients who underwent initial hepatectomy, a total of 214 (66%) experienced recurrence of their disease during the follow-up period of 44 months (median 36). Of these 214 patients, 179 (84%) had isolated intrahepatic recurrences. Re-resection of the liver was performed in 52 patients (29% of patients with intrahepatic metastasis) as well as another 8 patients who underwent initial hepatectomy at other hospitals **(Figure 2)**.

Outcome of Repeat Hepatectomies

Perioperative results: The mean and median blood loss for the 60 patients who underwent second hepatectomy was 584mL and 450mL, respectively (range, 46-2850mL). Two patients (3%) required BTF perioperatively. Surgery-related complications occurred in 20 patients (33%). There were no in-hospital

deaths.

Long-term outcome of second hepatectomy: The mean and median follow-up periods after repeat hepatic resection were 40 months and 39 months, respectively (range, 1-110 months). The 1-, 3-, and 5-year survival rates after the second hepatic resection were 96.7%, 73.9%, and 52.9%; disease-free survival rates at 1, 3, and 5 years were 60.4%, 18.7%, and 14.0%, respectively **(Figure 4)**.

Tumor recurrence after second hepatic resection: Recurrent hepatic disease after the second hepatectomy was found in 40 patients. Of these patents, 13 (33% of patients who had recurrence of their hepatic disease after the second operation) and another 5 patients who had their first and second liver resections at other institutions underwent a third liver resection **(Figure 2)**. The mean and median blood loss during the third liver resection was 746mL and 475mL, respectively (range, 60-2300mL). Surgery-related complications occurred in 4 patients (22%). Recurrence in the liver remnant after the third operation was confirmed in 11 patients, and a fourth operation was performed in two of them. There was no mortality in these patients who underwent third and fourth liver resections.

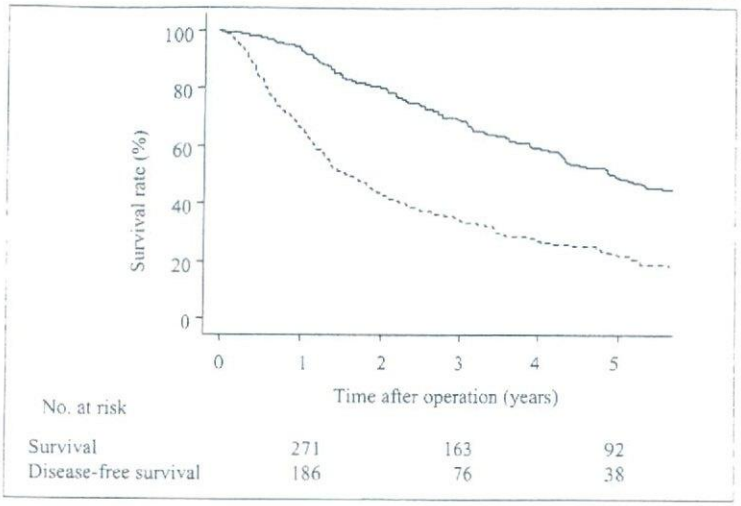

FIGURE 3 Cumulative survival (solid line; 1-, 3-, and 5-year survival rate = 93.1, 69.1, and 49.0%, respectively) and disease-free survival rate (dotted line; 1-, 3-, and 5-year survival rate = 66.9%, 34.8%, and 21.8%, respectively) after the initial hepatectomy in 324 patients with hepatocellular carcinoma.

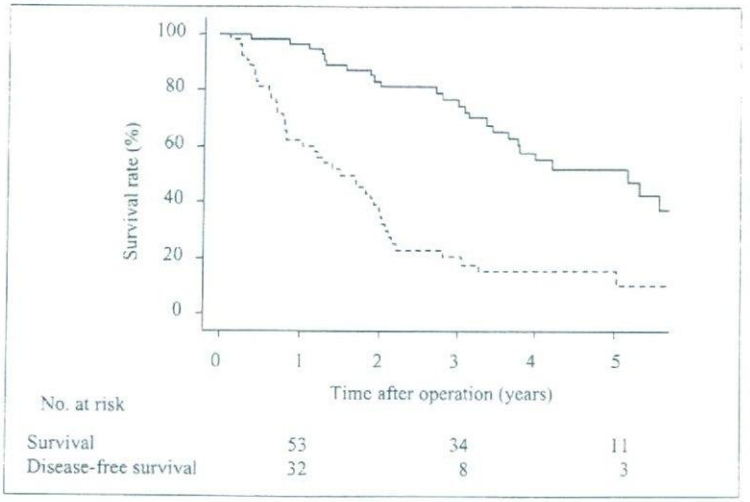

FIGURE 4 Cumulative survival (solid line; 1-, 3-, and 5-year survival rate = 96.7, 73.9, and 52.9%, respectively) and disease-free survival rate (dotted line; 1-, 3-, and 5-year survival rate = 60.4%, 18.7%, and 14.0%, respectively) after the second hepatectomy in 60 patients with recurrent hepatocellular carcinoma.

DISCUSSION

The aim of this study was to analyze our 11-year experience of liver resection in HCC patients and to clarify the role of hepatectomy as a treatment modality for HCC. One of the most notable findings was the low operative and hospital mortality rates. Several authors have reported that liver resection for HCC is becoming increasingly safe, with in-hospital mortality rates of 5-10% (1-3), and many surgeons appear to agree with these figures. In the present series, however, we performed 404 liver resections with close to zero mortality; i.e. 324 initial hepatectomies with one operative death (0.3%) and one hospital death (0.3%), respectively, and 80 repeat liver resections with zero mortality. Moreover, the mortality rate of patients with cirrhosis in previous studies seemed to be higher than that of patients without cirrhosis, ranging from 10% to 20% (16,17). In our study, 152 patients with liver cirrhosis underwent initial liver resection, with an operative mortality rate of only 0.7% (1/152). Our results seem to be considerably better than those reported previously, although these figures are not directly comparable because the patient population, the proportion of those undergoing "major" hepatectomy - defined as equal to and/or greater than hemihepatectomy - and disease stages differed from those reported by other institutions. In summary, liver resection for HCC can be performed with close to zero mortality. The 5-year survival rate of 53.2% after initial hepatectomy, which is comparable to those reported previously (18-20), is further supported by this low mortality rate.

Because most patients with HCC have associated chronic hepatitis or liver cirrhosis, both of which are major obstacles to performing liver surgery, it is vital to evaluate liver function when selecting patients for hepatectomy. Although many investigators rely on the Child-Pugh classification to estimate the hepatic functional reserves of surgical candidates (3,21), this classification was originally designed for prognosticating patients undergoing surgery for portal hypertension (22). Hence, it does not always indicate how much of the non-tumorous liver can be resected safely. Indeed, Bruix *et al.* (23) reported that more than 50% of patients with Child-Pugh class A disease had hepatic decompensation after liver surgery, and that this classification may provide only a rough evaluation of hepatic function. Our selection criteria for liver resection rely routinely on a scheme devised by Makuuchi and colleagues (8). Briefly, the type of resection is chosen on the basis of three factors: presence or absence of ascites, total serum bilirubin value, and the indo-

cyanine green retention rate at 15 min. Given the fact that operative and hospital mortality rates were low (0.3% each) in our series, our criteria can be regarded as sufficient for choosing the type of hepatectomy, and they seem to contribute greatly to the prevention of potentially lethal postoperative complications.

To date, several reports have investigated the association between BTF and the risk of recurrence after resection in patients with HCC (24-26). Interestingly, all of these reports have revealed an adverse effect of perioperative BTF on postoperative disease recurrence. Similarly, in our series, patients who received BTF had a significantly poorer prognosis than those who did not. Moreover, multivariate analysis revealed that BTF was a significant predictor of postoperative disease recurrence. Therefore, every effort should be made to avoid BTF by minimizing intraoperative blood loss; this is one of the few things the surgeon can do to reduce the rate of postoperative cancer recurrence. Despite improvements in surgical techniques, previous reports have shown that hepatic surgery frequently requires perioperative BTF. The blood transfusion rate in these reports was rarely less than 30% (24,26). In our series, however, only 9.9% of patients undergoing initial liver resection required BTF perioperatively. This low rate of BTF can be ascribed to the constant use of blood inflow occlusion by Pringle's maneuver or the hemihepatic vascular occlusion technique (10), meticulous surgical techniques to secure hemostasis (9), and our policy of avoiding perioperative BTF (14).

Unfortunately, the tumor recurrence rate after liver resection is still high, even if the operation is curative. Previous studies have described the cumulative 5-year recurrence rate as being in the 75-100% range (1,5,27), and in most cases recurrence occurs mainly in the remnant liver as a result of intrahepatic metastasis by the primary cancer and/or second primary tumor (5,28). Hence, effective treatment strategies for recurrent tumors are indispensable for improving the long-term outcome after resection of HCC. Repeat hepatectomy has been reported to be the treatment of choice for recurrent HCC within the remnant liver (29-31), although progression of underlying liver disease and a tendency toward tumor multiplicity at the time of recurrence seems to limit the indication for re-resection in a small group of patients. During the past 11 years, we have aggressively performed repeat hepatectomy (12,13) whenever possible in patients with recurrent tumors in their liver remnant. As a result, the rate of second and third resection reached 29% and 33% of all patients with isolated liver recurrence, respectively. These resection rates were attributed mainly to our strict follow-up after surgery. This aggressive surgical approach is supported by having performed these 80 repeat resections with no in-hospital deaths. In terms of long-term outcome, this study showed a 5-year survival rate of 52.9% after the second hepatectomy, which is comparable to those reported previously (29-31). In summary, repeat hepatectomy can be performed safely in patients with recurrent HCC and seems to be associated with prolonged survival time.

In conclusion, we have performed 404 consecutive liver resections for HCC with an in-hospital mortality rate close to zero and an overall 5-year survival rate of 53.2% after initial liver resection. These results confirm that hepatic resection with curative intent remains the mainstay of treatment for HCC. The safety of the procedure and its good long-term outcome might be attributed to the proper selection criteria for resection, the constant application of blood inflow occlusion and meticulous surgical techniques to reduce blood loss during resection, careful follow-up after surgery, and an aggressive surgical approach in patients with intrahepatic recurrence of HCC.

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Predictive factors for intrahepatic cholangiocarcinoma recurrence in the liver following surgery

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Editorial on page 925

Background. We performed hepatectomy without lymph node (LN) dissection for intrahepatic cholangiocarcinoma (ICC) limited to the peripheral region of the liver, and hepatectomy with extrahepatic bile duct resection and regional LN dissection for any types of ICC extending to the hepatic hilum. Surgical outcomes were evaluated to elucidate the prognostic factors that influence patient survival with respect to intrahepatic recurrence. ***Methods.*** Forty-one patients underwent resection of ICC with no macroscopic evidence of residual cancer. ***Results.*** Significant risk factors for poorer survival included preoperative jaundice ($P = 0.0115$), serum CA19-9 levels >37 U/ml ($P = 0.0089$), tumor diameter >4.5 cm ($P = 0.017$), ICC extending to the hepatic hilum ($P = 0.0065$), mass-forming with periductal-infiltrating type ($P = 0.003$), poorly differentiated adenocarcinoma, portal vein involvement ($P = 0.0785$), LN metastasis at initial hepatectomy ($P < 0.0001$), and positive surgical margin ($P = 0.023$). Intrahepatic recurrence, which was the predominant manner of recurrence, was detected in 20 patients (74.1%). Patients with intrahepatic recurrence had a significantly high incidence of high serum CA19-9 levels (>37 U/ml; $P = 0.0006$), preoperative jaundice ($P = 0.0262$), ICC extended to the hepatic hilum ($P = 0.0349$), large tumors (>4.5 cm; $P = 0.0351$), portal vein involvement ($P = 0.0423$), and LN metastasis at initial hepatectomy ($P = 0.009$) compared with disease-free patients. The multiple logistic regression analysis revealed that preoperative CA19-9 elevation and obstructive jaundice influenced intrahepatic recurrence of ICC. ***Conclusions.*** Although LN metastasis is a significant prognostic factor, the most obvious recurrence pattern after surgery was intrahepatic recurrence, which could be predicted preoperatively by a combination of elevated serum CA19-9 levels and manifestation of obstructive jaundice.

Key words: intrahepatic cholangiocarcinoma, intrahepatic recurrence, predictive factor, prognosis, lymph node metastasis

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Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary malignancy of the liver. ICC tends to invade the portal region directly, and to spread by perineural invasion, lymphatic involvement, and metastasis to local or distant lymph nodes (LNs).[1,2] Owing to its invasive and rapid growth properties, ICC is usually already at an advanced stage at the time of diagnosis. Curative resection remains the only effective treatment approach for ICC,[3–6] although the resectability rate is low and nonsurgical methods have failed to improve treatment success.[7] ICCs may exhibit different biological behaviors depending on both tumor location and morphology,[6,8,9] and are classified macroscopically into the following three types: mass-forming, periductal-infiltrating, and intraductal growth.[10] Among these categories, mass-forming tumors are the most common.

We have previously reported on the prognostic significance of matrix metalloproteinase-7 expression in ICC[11] and of the percentage of mature dendritic cells in ICC patients.[12] Several additional prognostic factors, including tumor size, surgical margin, intrahepatic metastasis, LN metastasis, vascular invasion, lymphatic invasion, and perineural invasion have also been reported.[13–18] However, the efficacy of LN dissection is uncertain, and Shimada et al.[19] reported minimal contribution of LN dissection to improvement of prognosis in