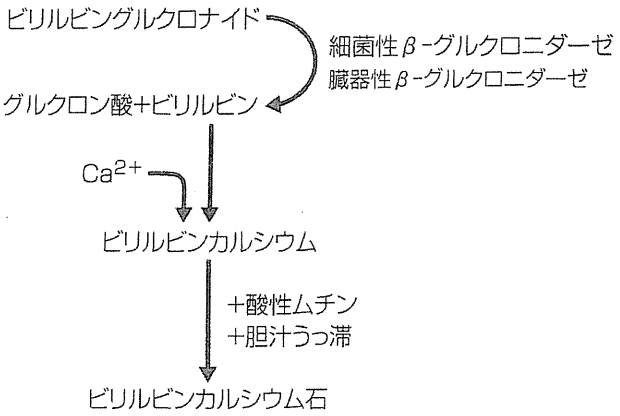


**図V-19　ビリルビンカルシウム石の形成機序**

橋作用によって凝集し，強固な結石様凝塊が形成され，これが徐々に成長してビリルビンカルシウム石になると考えられている(図V-19)[12]．

胆汁の生化学・生理学，胆石形成の分子機序について概説した．コレステロール胆石は，食生活習慣との関連性を有する結石症である．単なる胆嚢内のイベントではなく，肝臓や腸管における脂質代謝が深く関与する．これまでに蓄積されてきたエビデンスに基づき，今後，予防を視野に入れた病態研究のさらなる発展が望まれる．

◎文献

1) Hay DW, Carey MC：Pathophysiology and pathogenesis of cholesterol gallstone formation. Semi Liver Dis 10：159-170, 1990
2) Berge KE, Tian H, Graf GA, et al：Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290：1771-1775, 2000
3) Smith AJ, de Vree JM, Ottenhoff R, et al：Hepatocyte-specific expression of the human MDR3 P-glycoprotein gene restores the biliary phosphatidylcholine excretion absent in mdr2(-/-). Hepatology 28：530-536, 1998
4) Gerloff T, Stieger B, Hagenbuch B, et al：The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. J Biol Chem 273：10046-10050, 1998
5) Temel RE, Tang W, Ma Y, et al：Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J Clin Invest 117：1968-1978, 2007
6) Naureckiene S, Sleat DE, Lackland H, et al：Identification of HE1 as the second gene of Niemann-Pick C disease. Science 290：2298-2301, 2000
7) Yamanashi Y, Takada T, Yoshikado T, et al：NPC2 regulates biliary cholesterol secretion via stimulation of ABCG5/G8-mediated cholesterol transport. Gastroenterology 140：1664-1674, 2011
8) Lee SP, LaMont JT, Carey MC：Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones. J Clin Invest 67：1712-1723, 1981
9) Kibe A, Holzbach RT, LaRusso NF, et al：Inhibition of cholesterol crystal formation by apolipoproteins in supersaturated model bile. Science 225：514-516, 1984
10) 日本消化器病学会(編)：胆石症診療ガイドライン．南江堂，2009
11) 大薮久憲，田畑正久，中山文夫：胆汁中ビリルビンの非細菌性脱抱合．胆と膵 10：1335-1339, 1989
12) Maki T：Pathogenesis of calcium bilirubinate gallstone：role of E. coli, beta-glucuronidase and coagulation by inorganic ions, polyelectrolytes and agitation. Ann Surg 164：90-100, 1966
13) 新谷史明，伊勢秀雄，高橋良延，他：胆汁中ムコ物質．胆と膵 10：1335-1339, 1989

(正田純一)

# 総胆管結石症

choledocholithiasis

## 疾患概念

総胆管結石症と胆嚢結石症は同じ「胆石症」として扱われることが多いが，病態が異なるために，専門医としてはしっかりと区別してほしい．総胆管結石症は総胆管内に存在する結石による疾病である．定義上総胆管は解剖学的には胆嚢管合流部から乳頭までを指すが，臨床的には総肝管から乳頭部までの胆管に存在する結石は総胆管結石と称しているので，本項では後者の立場で解説する．

胆嚢結石とは異なり，有症状化が高率であることと，胆管炎発症時には重篤な状態に陥りやすいことから，無症状でも治療を検討する．胆管結石の発生に関しては胆嚢結石の落下(落下結石)と胆管内で発生する原発結石，数は少ないが肝内結石からの落下結石の3種類がある．落下結石の場合はコレステロール系結石や黒色石が多いが，原発結石では細菌感染と関連があり，ビリルビンカルシウム石が多い．

sy：EHL）などで結石を破砕した後に排石する（図112）.

b）経皮経肝的胆道鏡下治療

胃切除 Billroth II 再建などの経乳頭的アプローチが困難な例や総胆管充満結石などの内視鏡的砕石困難例，および肝内結石が対象となる．経皮経肝胆道ドレナージ（PTBD）でルートを作製し，瘻孔を拡張した後，胆管内に直接スコープを挿入して直視下にバスケットカテーテルなどを用いて砕石する．

c）外科療法

非観血的治療が困難な場合，総胆管切開切石術，胆道消化管吻合術，肝切除術などの外科治療が行われることがある．

〔菅野啓司，田妻　進〕

◉文献

1）日本消化器病学会胆石症検討委員会：日本における胆石の新しい分類．日消誌 1986；83：309.

2）Lammert F, et al：Gallstone disease. In：Rodes J, et al (eds). Textbook of Hepatology：From Basic Science to Clinical Practice, 3rd edition. New Jersey：Blackwell Publishing；2007, p.1518.

3）谷村　弘，内山和久：全国胆石症 1996 年度調査報告．胆道 1997；11：133.

# 胆道系の炎症

## 1●胆嚢炎 cholecystitis

### 概念

●胆嚢の炎症性疾患である．胆管炎と並んで胆道系の炎症性疾患の双璧をなすものであるが，頻度的には胆嚢炎が多い．発症要因として重要なものは胆石である．

●胆嚢炎はその臨症経過から急性と慢性，また，胆石の関与の有無により有石性と無石性に大別される．

●高齢者における胆石保有率は高く，かつ高齢者の胆道感染症は重症化しやすい．

● 2005 年には『科学的根拠に基づく急性胆管炎・胆嚢炎の診療ガイドライン』が策定され，さらに，2007 年には国際版ガイドライン "Tokyo Guidelines for the management of acute cholangitis and cholecystitis" も策定された．現在，新ガイドラインの改訂作業が進行中である．急性胆嚢炎に対する的確な診断，初期治療を行うための，治療方針と方法を決定するために必要な知識を得ることが重要である．

### 病因・病態生理

a 急性胆嚢炎

発生要因は何らかの原因により胆嚢内容が正常に排泄されなくなり，胆嚢内に胆汁がうっ滞することである．大部分は胆嚢内の胆石による胆嚢管の閉塞である．また，術後や長期中心静脈栄養（intravenous hyperalimentation：IVH）患者にみられる胆嚢収縮，弛緩をつかさどるホルモン刺激の欠如により胆汁がうっ滞して起こる無石性胆嚢炎がある．胆汁うっ滞による化学的炎症に細菌感染（起炎菌として，*Escherichia coli*，*Klebsiella*，*Enterobacter* などのグラム陰性桿菌が多い）が加わることにより，急性胆嚢炎としての典型的な病態の進展が起こる．

b 慢性胆嚢炎

胆石の存在する場合に多い．潜在的に炎症病態が進行することで慢性胆嚢炎として発症してくる場合と，頻回に胆道疝痛発作あるいは急性胆嚢炎に引き続いて発症する場合がある．後者が大部分を占める．

### 病理

a 急性胆嚢炎

胆嚢表面は光沢のない灰紅ないしは青緑色を呈し，炎症性滲出物で覆われている．胆嚢は腫大し，周囲臓器との癒着も認められる．胆嚢頸部に胆石が嵌頓していることが多い．組織学的には，急性期には出血および浮腫が生じる．急性期反応が消失すると線維性となる．頸部や総胆管周囲にはリンパ節の腫大が認められる．細菌学的には 70～80 %の症例で，胆嚢壁ならびに胆汁培養によって腸内細菌を証明する．

急性胆嚢炎は，病理組織学的に漿液性，化膿性，壊疽性胆嚢炎の 3 つに分類される．壊疽性胆嚢炎は重篤であり，しばしば胆嚢穿孔をきたす．特殊なものとして，急性気腫性胆嚢炎がある．*Clostridium perfringens* などのガス産生能を有する細菌感染によるものであり，胆嚢内腔，胆嚢壁，胆嚢周囲組織などにガス像を認めることがある（急性気腫性胆嚢炎，図 113）.

b 慢性胆嚢炎

胆嚢は萎縮し，壁は肥厚し，時には石灰化を伴う．粘膜面には潰瘍や瘢痕を伴う．組織学的には粘膜が肥厚し，リンパ球浸潤や粘膜破壊が認められる．

### 臨床症状

a 急性胆嚢炎

食後や夜間に発生し，右季肋部から心窩部痛，時に右背部から右肩甲骨部における放散痛が出現する．しばしば右上腹部にデファンスを認め，腫大した胆嚢を触知する．Murphy sign（右上腹部を手で圧迫しながら患者に深呼吸をさせると，疼痛のために吸気を途中で止める）は急性胆嚢炎の診断において，感度は 50～60 %であるが，特異度は 79～96 %と高い．

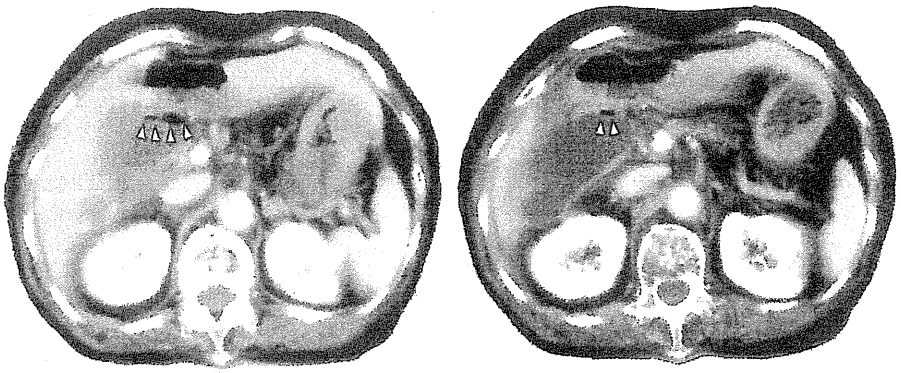

図 113 急性気腫性胆嚢炎の CT 画像
胆嚢壁の肥厚，胆嚢内腔（右）および胆嚢壁（左）のガス像を認めた．

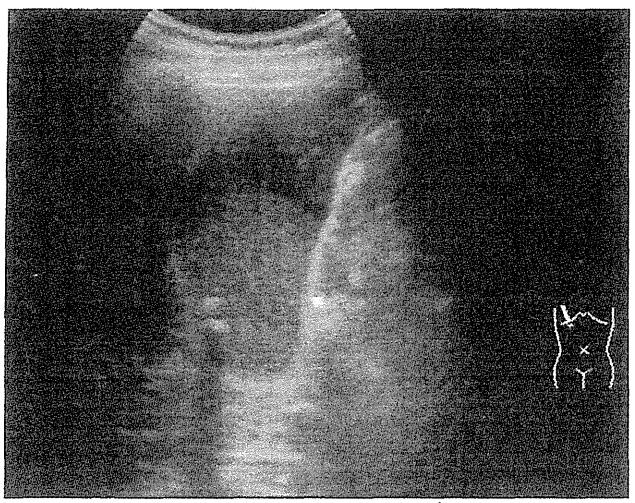

図 114 急性胆嚢炎の超音波画像
胆嚢腫大，胆嚢内デブリ，胆石像が観察される．

b 慢性胆嚢炎

無症状に経過したり症状に乏しい場合もあるが，多くは腹部膨満感，胃部不快感，右季肋部から心窩部鈍痛，また悪心，食欲不振，鼓腸などを訴える．

### 検査

a 急性胆嚢炎

血液検査では，白血球増多（左方移動を伴う）ならびに CRP 陽性の炎症所見が認められるが，生化学検査ではビリルビン，トランスアミナーゼ，胆道系酵素の軽度の上昇にとどまる．画像検査では腹部超音波検査が最も有用である（図 114）．胆嚢の腫大，胆嚢壁の肥厚（三層構造），胆泥（デブリ）の貯留が観察される．CT は胆嚢周囲膿瘍，胆嚢穿孔の診断に優れる．MRI は胆嚢頸部や胆嚢管結石の描出が良好である．

b 慢性胆嚢炎

血液生化学検査では，特徴的変化には乏しいことが多い．腹部超音波検査では胆嚢は腫大していることも萎縮していることもあるが，多くは胆石を伴っている．胆嚢壁はびまん性に肥厚し，三層構造を呈することもある．

### 診断

a 急性胆嚢炎

臨床症状，血液，画像検査の結果をふまえて，「ガイドライン」における診断基準（表 48）および重症度判定基準（表 49）と照らし合わせ，急性胆嚢炎の診断と重症度判定を行う．胆道感染症以外の疾患（急性膵炎，消化性潰瘍の穿孔，心筋梗塞など）の鑑別も重要である．

b 慢性胆嚢炎

上腹部不定愁訴に加えて胆石疝痛発作の既往を認めれば，本症である可能性が高い．胆嚢癌との鑑別が困難であることを念頭に検査を進める．

### 治療

a 急性胆嚢炎

「ガイドライン」に従い，急性胆嚢炎の場合は原則として胆嚢摘出術を前提とした初期治療（全身状態の改善と感染症治療）を行う．

初期治療として，緊急入院をさせ，絶飲食，輸液，抗菌薬，鎮痛薬の投与などの保存治療を行う．重症胆嚢炎（重篤な局所合併症を伴う症例，気腫性胆嚢炎，壊疽性胆嚢炎，化膿性胆嚢炎）では，全身管理を行いながら緊急で開腹手術が必要となる．

中等症例では，初期治療とともに迅速に手術や胆嚢ドレナージの適応を検討する．軽症例でも，初期治療（12～24 時間）に反応しない例では手術や胆嚢ドレナージの適応を検討する．高齢者の場合は，臨床上自他覚症状所見の発現が軽微なことが多いが，病勢が速やかに進展し，基礎疾患の増悪，続発症の併発をきたし，しばしば重篤な病態に移行しやすいため，早期の手術対応が必要である．

b 慢性胆嚢炎

胆嚢機能が保たれているものは，しばらく内科的に経過を観察する．胆石を伴い胆嚢機能が低下しているものや，急性増悪を繰り返すものでは，胆嚢摘出術の適応である．

表 48 急性胆嚢炎の診断基準

| | | |
|---|---|---|
| A. 臨床徴候 | 1. | Murphy sign |
| | 2. | 右上腹部腫瘤触知 |
| | 3. | 右上腹部痛か圧痛 |
| B. 炎症所見 | 4. | 発熱 |
| | 5. | CRP 値の上昇 |
| | 6. | WBC 数の上昇 |
| C. 画像所見 | | |

(1) A 項目の 1 つと B 項目の 1 つを認めたもの
(2) 臨床的に急性胆嚢炎が疑われ，C により確認されたもの
(＝A，B，C のうち 2 つ以上を満たしたもの，ということになる)
注) ただし，急性肝炎や，ほかの急性腹症，慢性胆嚢炎が除外できることとする．

CRP：C-reactive protein（C 反応性蛋白），WBC：white blood cell（白血球）．
(Tokyo Guidelines for the management of acute cholangitis and cholecystitis 2007)

### 予後

a 急性胆嚢炎

急性胆嚢炎の予後は，合併症の種類，年齢，他疾患(糖尿病や循環器系疾患)の存在によって左右される．多くの報告で急性胆嚢炎の死亡率は 10～11 %程度である．一般に無石性胆嚢炎のほうが有石性のものよりも予後不良とされている．

b 慢性胆嚢炎

胆嚢癌の存在が否定されて手術が施行されない場合には，厳重な経過観察が必要である．

## 2●胆管炎 cholangitis

### 概念

- 胆管炎は，胆管閉塞による胆道内圧の上昇に加えて，感染を合併した結果生じる胆管壁と内腔の炎症病態である．
- 原因としては，総胆管結石の嵌頓が多くを占めるが，悪性胆道閉塞を伴い発症する場合もある．
- 保存的治療で改善する軽症例から，敗血症，エンドトキシンショック，播種性血管内凝固（DIC），多臓器不全症候群などを合併して重篤な病態に陥る重症例までである．
- 2005 年には『科学的根拠に基づく急性胆管炎・胆嚢炎の診療ガイドライン』が策定され，さらに，2007 年には国際版ガイドライン "Tokyo Guidelines for the management of acute cholangitis and cholecystitis" も策定された．現在，新ガイドラインの改訂作業が進行中である．急性胆管炎に対する的確な診断，初期治療を行うための，治療方針と方法を決定するために必要な知識を得ることが重要である．

表 49 急性胆嚢炎の重症度判定基準

重症急性胆嚢炎
急性胆嚢炎のうち，以下のいずれかを伴う場合は "重症" である．
- 循環障害（ドパミン≧5 μg/kg/分，もしくはノルアドレナリンの使用）
- 中枢神経障害（意識障害）
- 呼吸機能障害（$PaO_2/FiO_2$ 比＜300）
- 腎機能障害（乏尿，もしくは S-CRE ＞ 2.0 mg/dL）
- 肝機能障害（PT-INR ＞ 1.5）
- 血液凝固異常（血小板＜100,000/μL）

中等症急性胆嚢炎
急性胆嚢炎のうち，以下のいずれかを伴う場合は "中等症" である．
- WBC 数＞ 18,000/μL
- 右季肋部の有痛性腫瘤触知
- 症状出現後 72 時間以上の症状の持続
- 顕著な局所炎症所見（胆汁性腹膜炎，胆嚢周囲膿瘍，肝膿瘍，壊疽性胆嚢炎，気腫性胆嚢炎）

軽度急性胆嚢炎
急性胆嚢炎のうち，"中等症"，"重症" の基準を満たさないものを "軽症" とする．

$PaO_2$：partial pressure of oxygen in arterial blood（動脈血酸素分圧），$FiO_2$：factional concentration of inspiratory oxygen（吸入〈気〉酸素濃度），S-CRE：serum creatinine（血清クレアチニン），PT-INR：prothrombin time-international normalized ratio（プロトロンビン時間国際標準比），WBC：white blood cell（白血球）．
(Tokyo Guidelines for the management of acute cholangitis and cholecystitis 2007)

### 病因・病態生理

胆管炎を発症するには，胆管の通過障害をきたす背景因子（狭窄や閉塞など）の存在が前提となる．胆管結石による通過障害が最も多いが，炎症性胆管狭窄，悪性腫瘍，寄生虫などがある．細菌の侵入経路としては，逆行性，血行性があるが，大部分は十二指腸乳頭を介した逆行性感染である．起炎菌は腸管内の常在菌である（*Escherichia coli*，*Klebsiella*，*Enterobacter*，*Bacteroides* など）．混合感染もみられる．胆管閉塞による胆管内圧の上昇が生じ，cholangiovenous reflux が惹起されることにより，汚染胆汁が血中に逆流し，菌血症，敗血症，エンドトキシン血症をきたす．さらに，DIC や多臓器不全を併発し，胆管炎の重篤化につながる．

### 臨床症状

急性胆管炎の典型的な症状は発熱，上腹部痛，黄疸の Charcot 三徴である．Charcot 三徴に加えて，ショック，意識障害の二徴が加わった場合を Reynolds 五徴と称する．「ガイドライン」に示されている重症急性胆管炎は重篤であり，五徴を示す頻度が高い．

### 検査・診断

表 50 に「ガイドライン」における診断基準，表 51 に重症度判定基準を示す．急性胆管炎の診断には

表 50 急性胆管炎の診断基準

| | | |
|---|---|---|
| A. 臨床徴候 | 1. | 発熱（悪寒，戦慄を伴う場合もある） |
| | 2. | 黄疸 |
| | 3. | 腹痛（右季肋部または上腹部） |
| | 4. | 胆道疾患の既往歴 |
| B. 検査所見 | 5. | 炎症所見（WBC 数，CRP の上昇，その他の炎症所見） |
| | 6. | 肝機能異常（ALP，γ-GTP，AST，ALT の上昇） |
| C. 画像所見 | 7. | 胆管拡張，成因となる所見（狭窄，結石，ステントなど） |

確診：(1) Charcot 3 徴（2＋3＋4）
(2) A の 2 つ以上かつ B と C の両方を満たすもの
疑診：A の 2 つ以上

WBC：white blood cell（白血球），CRP：C-reactive protein（C 反応性蛋白），ALP：alkaline phosphatase（アルカリホスファターゼ），γ-GTP：γ-glutamyl transferase（γ-グルタミルトランスフェラーゼ），AST：aspartate amino transferase（アスパラギン酸アミノトランスフェラーゼ），ALT：alanine amino transferase（アラニンアミノトランスフェラーゼ）．

（Tokyo Guidelines for the management of acute cholangitis and cholecystitis 2007）

表 51 急性胆管炎の重症度判定基準

重症急性胆管炎
急性胆管炎のうち，以下のいずれかを伴う場合は“重症”である．
- 循環不全（ドパミン≧5 μg/kg/分，もしくはドブタミンの使用）
- 神経学的異常（意識障害）
- 呼吸不全（$PaO_2/FiO_2$ 比＜300）
- 腎機能障害（S-CRE＞2.0 mg/dL）
- 肝機能障害（PT-INR＞1.5）
- 血小板＜100,000/μL

中等症急性胆管炎
臓器不全はないが初期治療に反応しなかった急性胆管炎を“中等症”とする．

軽度急性胆管炎
初期治療に反応した急性胆管炎を“軽症”とする．

$PaO_2$：partial pressure of oxygen in arterial blood（動脈血酸素分圧），$FiO_2$：factional concentration of inspiratory oxygen（吸入〈気〉酸素濃度），S-CRE：serum creatinine（血清クレアチニン），PT-INR：prothrombin time-international normalized ratio（プロトロンビン時間国際標準比）．

（Tokyo Guidelines for the management of acute cholangitis and cholecystitis 2007）

Charcot 三徴が用いられてきたが，本症の 50 ～ 70 % 程度を診断するにとどまっていることから，これを補うために，この三徴（発熱，腹痛，黄疸）のいずれかに加えて，ALP または γ-GTP の上昇，白血球増多または CRP 上昇，画像所見（胆管拡張，狭窄，結石）を満たすものも急性胆管炎と診断し，精度が向上している．重症度判定に関して「ガイドライン」では，急性胆管炎のなかでも臓器不全を伴うものを重症とし，臓器不全はないが速やかにドレナージを必要とするものを中等症とし，それ以外を軽症としている．搬送基準として，重症度判定後に，特に重症あるいは重症化が予想される場合には，胆道ドレナージなどの治療や患者管理ができないと判断された場合には搬送を考慮する．

### 治療

#### a 緊急処置

応急処置として，絶食，輸液，抗菌薬投与，全身状態の評価が必要である．潜在的な脱水状態を回避するために，胆道ドレナージに先立って十分な輸液を行う．

#### b 胆道ドレナージ

胆道ドレナージは，急性胆管炎の原因である胆汁うっ滞を解除する根本的な方法であり，本症治療の中心となるものである．結石の場合は排石よりもドレナージを優先させ，胆管内の圧を軽減させ，膿汁を排液させる．胆道ドレナージ術の施行経路には，①内視鏡的，②経皮経肝的，③手術的な方法が報告されているが，①，②，③の順に推奨されている．ドレナージの時期に関しては，重症度判定基準に基づき，重症例では緊急に，中等症では初期治療とともに速やかに，軽症例では初期治療（24 時間以内）に反応しない場合に施行する．

#### c 抗菌薬投与

原則的に全症例に full dose の抗菌薬を静注にて投与する．抗菌薬の適応基準も重症度に基づくべきであり，軽症にはペニシリン系薬剤や第一世代セフェム系薬剤を用い，中等症，重症には第二～四世代セフェム系薬剤を第一選択薬とし，ニューキノロン系薬剤，カルバペネム系薬剤を第二選択薬として用いる．

### 予後

重症急性胆管炎では，死亡率は 30 % となる．

## 原発性硬化性胆管炎

primary sclerosing cholangitis（PSC）

### 概念

- 原発性硬化性胆管炎（PSC）は，肝外および肝内胆管のびまん性の慢性炎症性疾患で，進行に伴い胆管の線維化により狭窄や閉塞をきたし，胆汁の流出障害を起こす．
- 診断基準（表 52）の除外項目にあるような二次性のものを除いた原因不明のものを指す．

### 疫学

全国アンケート調査（2003 年）では，本症 388 例の年齢分布は 20 歳代と 60 歳代に二峰性のピークが認められる．炎症性腸疾患の合併も若年者に多い．

男女比は，2003 年の調査では男性が 59 % とやや男性優位である．発症頻度は，年間数十例と推定される．

表 52 原発性硬化性胆管炎（PSC）の診断基準（Mayo クリニック，2003）

1. あらゆる部位の胆管に生じた典型的な胆管造影の異常所見
2. 臨床像（IBD の病歴，胆汁うっ滞の症状）および血液生化学データ（6 か月以上にわたり ALP が 2～3 倍に上昇）が合致
3. 二次性硬化性胆管炎の明らかな原因の除外
   a. 胆管炎
   b. AIDS の胆管障害
   c. 胆管悪性腫瘍（PSC 診断後は例外）
   d. 胆道の手術，外傷
   e. 総胆管結石
   f. 先天性胆道異常
   g. 腐食性硬化性胆管炎
   h. 胆管の虚血性狭窄
   i. floxuridine（未承認薬）動注による胆管障害や狭窄

（Lindor KD, et al : Primary sclerosing cholangitis. In : Schiff ER, et al 〈eds〉. Schiff's Diseases of the Liver, 9th edition. Lippincott Williams & Wilkins ; 2003, p.673.）

## 病因

原因は不明であるが，自己免疫の関与が考えられている．欧米では炎症性腸疾患の合併が多いために病因との関連が指摘されている．

## 病理・病態生理

PSC では，リンパ球浸潤を伴う炎症所見と著明な線維増生が肝内および肝外胆管周囲に認められる（胆管周囲の線維化〈periductal fibrosis〉，図 115）．この線維増生により胆管は狭小化し，その末梢側胆管は管腔の拡張をきたす．肝組織は慢性の胆汁うっ滞の所見を呈する．

## 臨床症状

主な症状として，黄疸，右上腹部痛，皮膚瘙痒感，体重減少，全身倦怠感などが高頻度に認められる．さらに，胆道感染を伴うと発熱をみる．

## 検査・診断

Mayo クリニックから提案されている診断基準案について最新のものを表 52 に示す．

### a 胆管造影の所見

診断上最も重要であり，特徴的な胆管変化（beaded appearance，図 116）が肝内および肝外胆管に認められる．胆管造影所見は基本的には ERCP による．

### b 臨床像と血液生化学データ

胆汁うっ滞の所見として，ALP，γ-GTP などの胆道系酵素の上昇が認められる．ただし，ALP の上昇は本症の 34 %では正常上限の 2 倍未満であることから除外の対象にはならない．また，わが国における炎症性腸疾患の合併は欧米より少なく，炎症性腸疾患の合併がなくても除外とはならない．胆汁うっ滞の症状も必発ではない．

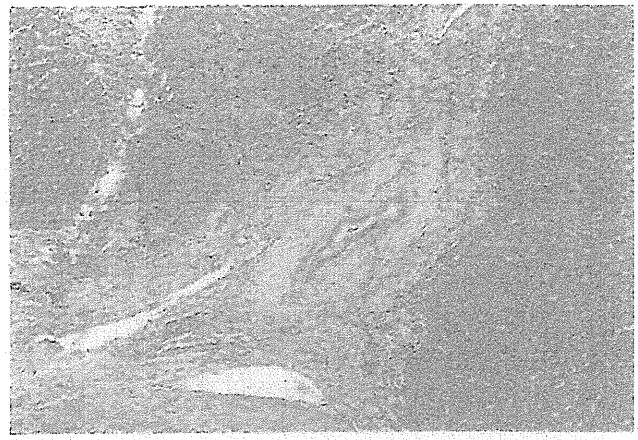

図 115 原発性硬化性胆管炎の組織像（HE 染色）
胆管周囲の線維化（periductal fibrosis）が認められる．

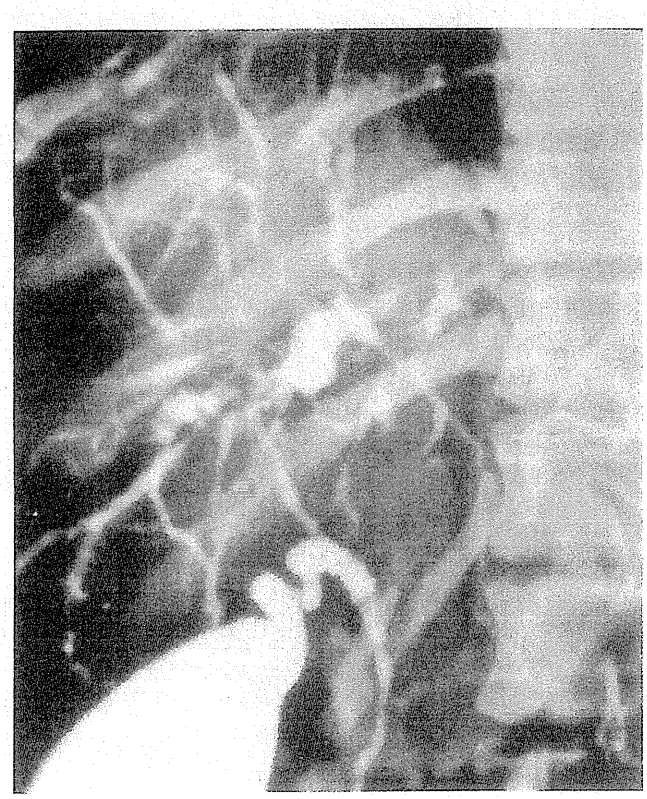

図 116 原発性硬化性胆管炎の ERCP 像
beaded appearance が認められる．

### c 二次性硬化性胆管炎の除外

除外項目として重要である．最近，いわゆる自己免疫性膵炎，膵管狭小型膵炎を合併した硬化性胆管炎は，副腎皮質ステロイドが著効し予後も良好であることより，PSC とは別の範疇の疾患として取り扱うべきであるとの見方が主流となっている．

## 合併症

潰瘍性大腸炎，後腹膜線維症，膵炎，Sjögren 症候群などがある．欧米では PSC に高率に潰瘍性大腸炎を合併すると報告されているが，わが国では欧米ほど高率ではない．また，PSC では約 10 %に胆管癌を合併することから，診断がついた後でも注意深く検索することが重要である．

### 治療

PSCに対する特異的ならびに根本的治療は現在のところ確立されていない．薬物療法として，胆汁酸製剤のウルソデオキシコール酸（UDCA）が最もよく使用され，胆汁うっ滞所見の改善に有用であるが，長期予後の改善に関しては議論が分かれる．副腎皮質ステロイドも有用であるとの報告があるが，副作用のために長期投与は難しい．胆管狭窄部に対する内視鏡的バルーン拡張術や金属ステント挿入は効果を上げている．進行した症例では肝移植が適応となる．

### 経過・予後

一般的には進行性で予後不良であり胆汁性肝硬変へ移行する．肝不全に至った症例では，肝移植が唯一の救命方法である．PSCには胆道癌を合併する．その合併率は欧米において7～18％，わが国において3.6％と報告されている．PSCに胆道癌を合併した症例は進行例が多く予後もきわめて不良であり，胆道癌はPSCの重要な予後規定因子である．

〔正田純一〕

◉文献
1) 急性胆道炎の診療ガイドライン作成委員会（編）：急性胆管炎・胆嚢炎ガイドライン（第一版）．東京：医学図書出版；2005.
2) Takada T, et al：Tokyo Guidelines for the management of acute cholangitis and cholecystitis. *J Hepatobiliary Pancreat Surg* 2007；14：1.
3) Lindor KD, et al：Primary sclerosing cholangitis. In：Schiff ER, et al (eds). Schiff's Diseases of the Liver, 9th edition. Philadelphia：Lippincott Williams & Wilkins；2003, p.673.
4) Lazaridis KN, et al：Primary sclerosing cholangitis and cholangiocarcinoma. *Semi Liver Dis* 2006；26：42.

## 胆道系の腫瘍

### 1●胆嚢癌 gallbladder cancer

#### 概念

●胆嚢および胆嚢管に原発する癌腫をいう（図117）．

#### 病因・疫学

胆石症は胆嚢癌の40～60％に認められる．一方，胆石症の胆嚢癌合併率は2～5％と胆石非保有例の0.4～1.0％と比べ高い．胆石症と胆嚢癌の密接な関連が推測されている．その機序として，胆石による機械的刺激，胆汁変化による化学的刺激，合併胆嚢炎による炎症性刺激などが想定されている．

膵・胆管合流異常症の16％が胆嚢癌を合併し，これは先天性胆道拡張例，胆道非拡張例のいずれにもみられる．合流異常に基づく膵液の胆道内逆流が発癌に関与していると考えられる．

男女比は1：2で，60～70歳代に多い．

#### 病理

胆嚢癌の80～90％は腺癌で，腺扁平上皮癌や扁平上皮癌もみられる．肉眼型から乳頭型，結節型，平坦型に分けられ，さらに膨張型と浸潤型に亜分類される（図118）．さらに充満型（胆嚢は腫瘍に充満されるが原型をとどめる），塊状型（原型をとどめず肝浸潤が高度）も含める．

癌の深達度は粘膜，固有筋層，漿膜下層，漿膜に分けられる．早期癌は，癌が粘膜や固有筋層内にとどまるものである．癌が漿膜下層に達してもRokitansky-Aschoff洞（RAS）にとどまる場合は早期癌とする．早期癌ではリンパ節転移はほとんどみられない．

胆嚢癌の進展様式として，粘膜内進展（胆嚢管・胆管浸潤），漿膜浸潤（胆嚢壁は粘膜筋板を欠くので容易に浸潤する），肝床（胆嚢床）部肝内直接浸潤，肝門浸潤・肝十二指腸間膜浸潤（間質浸潤，神経周囲浸潤），リンパ節転移（肝十二指腸間膜内，膵頭後部，総肝動脈幹，大動脈周囲），血行性転移（肝），腹膜播種などがあげられる．

#### 臨床症状

初期には無症状か，合併する胆石や胆嚢炎による症状を認める．進行すると右季肋部から心窩部に不快感

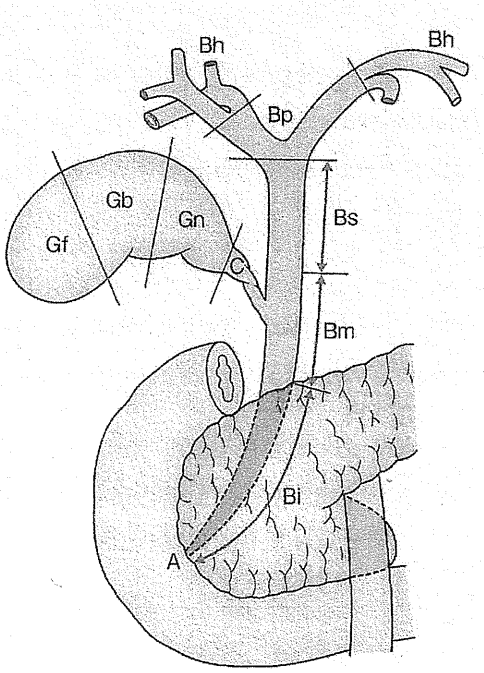


図117 肝外胆道系の区分

Bh：肝内胆管，Bp：肝門部胆管，Bs：上部胆管，Bm：中部胆管，Bi：下部胆管，Gf：胆嚢底部，Gb：胆嚢体部，Gn：胆嚢頸部，C：胆嚢管，A：乳頭部．

（日本胆道外科研究会〈編〉：胆道癌取扱い規約，第5版．金原出版；2003, p.3.）

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*Chapter 1*

# GALLBLADDER CANCER: PATHOGENESIS AND MOLECULAR TARGETING STRATEGIES FOR THERAPEUTIC OPTIONS

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## SUMMARY

Gallbladder cancer is a lethal malignancy. This is essentially attributed to slow carcinogenesis under the complex of pathological circumstances and to asymptomatic growth of gallbladder cancer infiltrating the surrounding structures in varying routes. The disease is therefore usually detected at an advanced stage with a high frequency of distant organ metastasis. To date, conventional chemotherapy and radiation therapy have been notably ineffective against gallbladder cancer. For improved treatment outcome of gallbladder cancer and its prolonged survival, there is now a real and urgent need to focus on developing novel and potent therapeutic strategies aimed at exploiting select molecular targets associated with tumor proliferation, invasion and/or metastasis that would impact in a significant way on clinical outcome. The outcome of recent studies, by the analysis of gallbladder cancer cells, animal models of gallbladder cancer, and clinical specimens of patients with gallbladder cancer, has revealed in detail the molecular mechanism of carcinogenesis and tumor progression of gallbladder cancer, and has exploited select molecular targets that would have a significant impact on clinical outcome. In the USA and European countries, only a few clinical phase II trials against gallbladder cancer and other biliary tract carcinoma using anticancer drugs targeting growth factor receptors have been reported. Development of new molecular targeting drugs with potent efficacy against gallbladder cancer and randomized clinical trials of these drugs are urgent and essential for the treatment of patients with gallbladder cancer.

## INTRODUCTION

Nearly two thirds of biliary tract cancers arise in the gallbladder, making gallbladder cancer the most common biliary tract cancer. Gallbladder carcinoma has always been associated with a dismal overall prognosis [1,2]. This is essentially attributed to the slow and asymptomatic growth of gallbladder carcinoma infiltrating surrounding structures, and the disease is therefore usually only detected at an advanced stage with a high frequency of distant organ metastasis [3-5]. In some cases, the disease is likely to undergo metastasis to the peritoneum (peritoneal dissemination) or the liver at early stages [6,7]. Details of tumorigenesis as well as growth and progression of the disease are complex and not completely understood.Thus, gallbladder cancer has been viewed as intractable cancers unlikely to be cured completely. In Japan, the incidence of biliary tract cancer including gallbladder cancer is about 10 out of every 100,000 people [8], which is higher than the incidences in other countries. Among others, these cancers in females are expected to exceed gastric cancer and breast cancer in the next ten years in terms of the death rate, thus becoming the fifth leading cause of death from cancer of various organs [9]. Furthermore, the annual number of deaths from biliary tract cancer is approximately equal to the annual number of patients newly diagnosed as having this cancer, indicating that biliary tract cancer is a type of cancer with considerably poor prognosis.

Gallbladdercancer is often resistant to conventional chemotherapy and radiotherapy. In Japan, gemcitabine and S-1 have begun to be used for anticancer chemotherapy [10-13], and these drugs are expected to prolong the survival period of patients as compared to existing anticancer drugs. Moreover, it has recently been reported that cisplatin plus gemcitabine is associated with a significant survival advantage as compared with gemcitabine alone [14]. However, because of frequent adverse events of the hematological system arising from these drugs and because of compromised hepatic function often noted in patients with gallbladder cancer due to accompanying obstructive cholestasis, treatment with these drugs has to be discontinued.

To improve the outcome of treatment of gallbladder cancer, it is very important to identify the tumor biological factors involved in the various progression waysof the disease(e.g., infiltration and metastasis) and to develop new valid means of auxiliary treatment targeted at these factors (those means are expected to manifest high efficacy while minimizing invasiveness to the host). This paper outlines the molecular mechanisms of onset and progression of biliary tract cancer including gallbladder cancer and refers to the perspectives for molecular-targeted therapy for the disease expected in the future.

## EPIDEMIOLOGY AND RISK FACTORS FOR GALLBLADDER CANCERS

Gallbladder cancer is a relatively rare neoplasm that differs from other cancers of the digestive tract, as being several-fold more common in women than in men [15].The incidence has considerable geographic and ethnic variations [16,17].

Sex-specific incidence rates of gallbladder cancer and female-to-male ratios for the most recent years available are available worldwide. The highest incidence rate was shown for women from Delhi, India (21.5/100,000), followed by South Karachi, Pakistan

(13.8/100,000) and Quito, Ecuador (12.9/100,000). High gallbladder cancer incidence rates were also found in cancer registries from Far East Asia (Korea and Japan), Eastern Europe (Slovakia, Poland, Czech Republic and Yugoslavia) and South America (Colombia). In Western Europe, elevated incidence rates were seen in Granada, Spain. High incidence rates among men (ranging between 4.4 and 8.0) were found in some areas of Asia and Eastern Europe. Low incidence rates (below 3/ 100,000 women and 1.5/100,000 men) emerged for most registries from Northern Europe, with the partial exception of Sweden, and from the USA (SEER) and Canada.

In detail, in the United States, ~5000 new cases of gallbladder cancer are diagnosed per year [18]. Japan has one of the world's highest age-adjusted cancer death rates related to gallbladder cancer, and it appears to be steadily increasing [9]. In 2007, biliary tract cancer including gallbladder cancer became the sixth leading cause of death from cancer of various organs and ca.17,000 subjects died per yearof the disease [8].

A systematic review of published findings on the association of gallbladder cancer with the potent risk factors is recently available and can address the above issue [19]. The consistent associations were found to be present with a history of gallstones, obesity, multiparity and chronic infections like Salmonella typhi, S. paratyphi, Helicobacter bilis, and H. pylori.

The geographic and ethnic variations in the incidence of gallbladder cancer can have several interpretations, but they refer particularly to the worldwide distribution of gallstones, which are the most important risk factor for gallbladder cancer [20,21]. The association between a history of benign gallbladder diseases (mainly gallstones) and gallbladder cancer risk was evaluated, and the relative risk for a history of gallstones was found to be significantly heterogeneous among the studies, such as cohort studies [22-24] and case-control studies [21,25-30]. Family history of gallbladder disease was also associated with an increased risk of gallbladder cancer in 2 studies [25,31]. Moreover, the particularly strong association between gallbladder cancer risk and large gallstones (>3 cm) was confirmed in 2 studies [32,33].

In addition, the results ofthe Japan Public Health Center-Based Prospective Study, an epidemiological study based on the prospective follow-up of a large number of the Japanese population [34] has recently been reported from the Ministry of Health, Welfare, and Labor, and has concluded that the past history of gallstone disease is proven to be a risk factor for gallbladder cancer. However, only a small proportion (1–3%) of patients with gallstones develop gallbladder cancer [35],and other risk factors have been proposed to play a role. The interplay of risk factors for gallbladder carcinogenesis; e.g., genetic susceptibility, lifestyle factors, and infections; is still poorly understood.

Obesity and overweight are major risk factors for gallstones [36], and body mass index (BMI) was related to gallbladder cancer risk in the previous studies [21,37-43]. In a cohort study [37], the association of GC with obesity was the second strongest among all cancer sites after that with cancer of the corpus uteri among women and liver cancer among men. In respect to dietary factors, in the multinational collaborative study from the SEARCH program, the strongest direct associations with gallbladder cancer risk were for total carbohydrate intake and total energy intake, and the inverse ones were for fibers, vitamin V6, E and C [21]. However, apart from obesity, there is no nutritional or dietary factor consistently related to gallbladder cancer risk.

High parity and number of pregnancies, again recognized risk factors for gallstones, were related to increased gallbladder cancer risk in the majority of the 10 studies dealing with this association, depending on the level of parity and gravidity considered [21,26,39,42,44-47].

Chronic infection of the gallbladder may contribute to the onset of gallbladder cancer, per se or via gallstone formation. Most available evidence implicates S. typhi and S. paratyphi. Epidemiological studies [24,42,43,46,48-51] have shown the relation between S. typhi and S. paratyphi and gallbladder cancer. All studiesbased on biological markers, such as serum Vi antigen or thepresence of the bacteria in bile specimens, found a significant positiveassociation between the carrier state of S. typhi and S. paratyphiand gallbladder cancer risk.

H. bilis and H. pylori have been identified in bile specimens and associated with risk of biliary tract cancer [52-54]. One study [53] conducted in Japanese and Thai populations showed that patients positive for H. bilis had an ~6-fold higher risk of biliary tract carcinoma than the negative ones. H. pylori infection was also identified as a risk factor for biliary tract cancer [53]. Another study [55] found a positive association between H. bilis and gallbladder cancer.Unfortunately, most studies of infection and GC to date have been small (no more than 15 exposed cases), have lacked well matched controls (with or without gallstones) and have been hampered by the lack of standardized and non invasive methods (from self-reported diagnosis to PCR) for the detection of these infectious agents in large epidemiological studies.

Collecting the findings of the published findings on the association of gallbladder cancer with the potent risk factors, it seems that a main pathway to gallbladder cancer exists worldwide and that the predominant pathway involves gallstones and resultant cholecystitis and affects women to a greater extent than men [56].

## MOLECULAR MECHANISMS OF CARCINOGENESIS AND CANCER PROGRESSION

In a clinical aspect, for many years, chronic inflammation of the biliary tract epithelium has been thought to be involved in the mechanisms of cancer onset and progression of biliary tract cancer for the following reasons: (1) Gallbladder cancer is often complicated by gallstones or chronic cystitis [19,57]. (2) Intrahepatic bile duct cancer develops in the segment of the bile duct close to the area affected by primary sclerosing cholangitis, liver flukes, or stones (causing chronic proliferative cholangitis associated with intrahepatic lithiasis) [58,59]. (3) Chronic biliary tract inflammation accompanied by metaplastic or dysplastic changes are noted in the cancer-free mucosal epithelium around the gallbladder cancer. (4) Experimental induction of inflammation in the biliary tract epithelium leads to hyperplastic or dysplastic changes and an increase in cell proliferative potentials.Taken together, at any site the of biliary tract (e.g., gallbladder, extra- or intrahepatic bile duct), the long-standing injuries of the epithelia due to bile stasis and chronic inflammation, that are coupled with abnormalities in epithelial restoration and repair mechanism, may play a certain role in stimulating the process of carcinogenesis and cancer progression.

In normal cells, cellular signal transduction pathways transmit information from one molecule to another and eventually regulate the function of genes located in the nucleus. Multiple signal transduction systems interact with each other to control cell proliferation,

apoptosis, function/secretion, etc., thus preserving the orderly number and function of normal cells. If a chronic inflammatory disease or other factors described later cause abnormalities in genes encoding some particular molecules involved in cellular signal transduction pathways, the relevant signal transduction systems become abnormal, resulting in the inability to control cell proliferation or persistence of the cells originally destined to lose viability. This might lead to the appearance of cancer cells.

Usually, cancer cells are not present independently but are located in microenvironments composed of normal host cells (e.g., parenchymal cells, interstitial cells, etc.) and extracellular matrix. For the expression of factors related to biliary tract carcinogenesis, the importance of host cells (playing a secondary role) in addition to that of cancer cells (playing the primary role) has been indicated. To be more precise, in the presence of an inflammatory disease, physiologically active substances (growth factors, cytokines, prostaglandins, etc.) are produced in the microenvironments composed of host cells and extracellular matrix; changes in these micro-environmental factors due to chronic exposure to bile acid activate oncogenes and cause abnormalities in cellular signal transduction pathways, leading to the induction of cancer cells (Figure 1). In addition, changes in these factors also modify the expression of factors related to infiltration and metastasis of cancer.

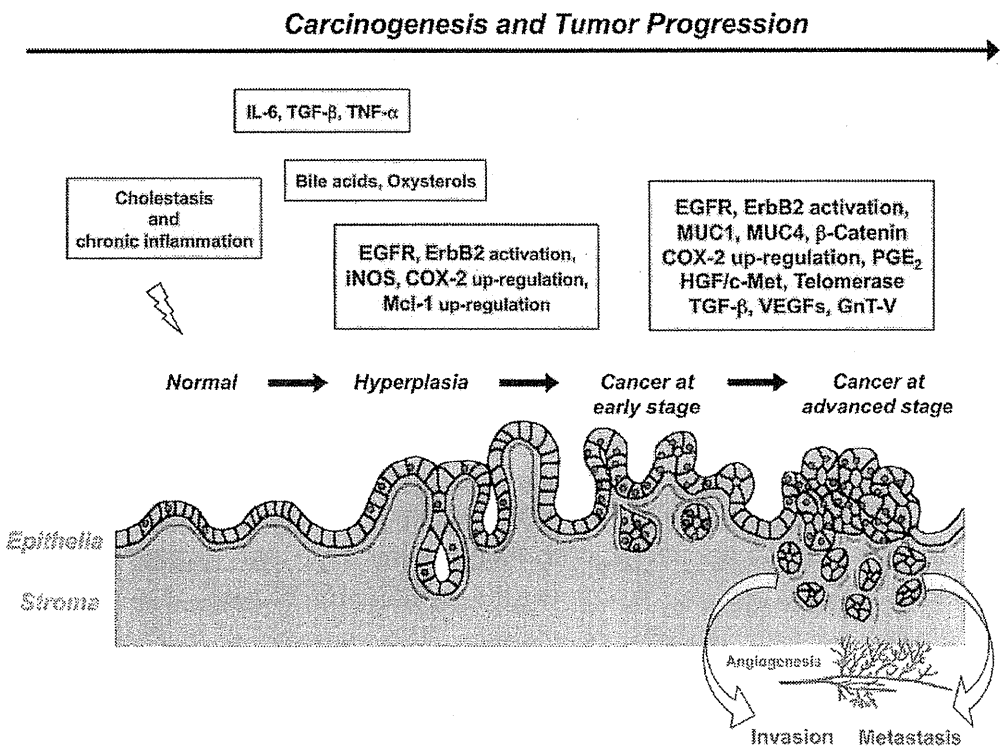


Figure 1. A schematic overview of key mechanisms regulating gallbladder carcinogenesis.

Sirica recently elegantly outlined the molecular mechanisms of carcinogenesis and cancer progression of cholangiocarcinoma (bile duct cancer) in a review paper, focusing on potential tumor-associated molecules useful for chemical prevention and molecular-targeted therapy for cholangiocarcinoma [60]. Since these tumor-associated molecules might also play central roles in the onset and progression of gallbladder cancer, that paper is partially presented below.

## MOLECULAR MECHANISM OF CARCINOGENESIS

Bile tract cancer is usually known to develop from the epithelium of gallbladder, extrahepatic or intrahepatic bile ducts through multiple steps [61,62]. Underlying pathologic conditions are disorders of the biliary epithelium due to bile stasis (primarily involving bile components such as bile acid) associated with chronic inflammation and obstructive cholestasis. The tumor biological molecules involved in biliary tract carcinogenesis as target molecules for chemoprevention include tumor necrosis factor-alpha (TNF□), inducible nitric oxide synthetase (iNOS), cyclooxygenase-2 (COX-2), epidermal growth factor (EGF) receptor, myeloid cell leukemia protein 1 (Mcl-1), etc. and interactions among these molecules [60,63-66]. Inflammatory cytokines produced in the presence of inflammation induce the expression of iNOS, leading to excessive formation of NO in the biliary tract. This causes disorders of the biliary epithelium due to oxidative stress. The increase in DNA damage and suppression of DNA repair stimulate gene mutation [67]. Induced iNOS and oxysterols contained in bile induce COX-2, resulting in increased formation of prostaglandin E2 (PGE2) due to activation of the arachidonic acid metabolism and leading to acceleration of cancer cell proliferation, accompanied by prolongation of the survival period of cancer cells. Furthermore, bile acid induces transactivation of EGF receptor, resulting in the induction of COX-2 and the suppression of Mcl-1 (anti-apoptosis protein) degradation, which leads to suppression of apoptosis. Due to accumulation of these intracellular events, biliary tract carcinogenesis is probably stimulated. Therefore, the molecules involved in these events may be useful as target molecules for chemoprevention of biliary tract cancer, including gallbladder cancer.

Recently, a couple of papers [64,68] have shown that interleukin-6 (IL-6), an inflammatory cytokine, may play a significant role in the onset and progression of bile duct cancer by causing a chronic inflammatory pathological condition in the biliary tract. IL-6 is produced not only in interstitial cells but also in cancer cells. The produced IL- 6 binds the IL-6 receptors on the tumor cell surface and then activates several signaling pathways; e.g., JAK/STAT, PI3/Akt, and MAPK (Figure 2). Activation of the JAK/STAT pathway leads to nuclear translocation of a transcription factor STAT3 and then to an induction of Mcl-1. This pathway activation also up-regulates EGFR expression in cancer cells. The activation of the PI3/Akt pathway inhibits apoptosis and thereby induces cell growth promotion. The activation of the MAPK pathway promotes cell proliferation through a modification of cell cycle-related gene expressions [69]. In addition, IL-6 is involved in the control of DNA methyltransferase expression and mediates methylation of several genes that are important for biliary carcinogenesis [68].

Excessive expression of these tumor biological molecules and activation of interactions among these molecules probably stimulate the progression of biliary tract cancer. Treatment targeted at these molecules may suppress the progression of cancer through the inhibition of the malignant behavior of cancer cells. The development of new drugs for various target molecules is desirable in the future.

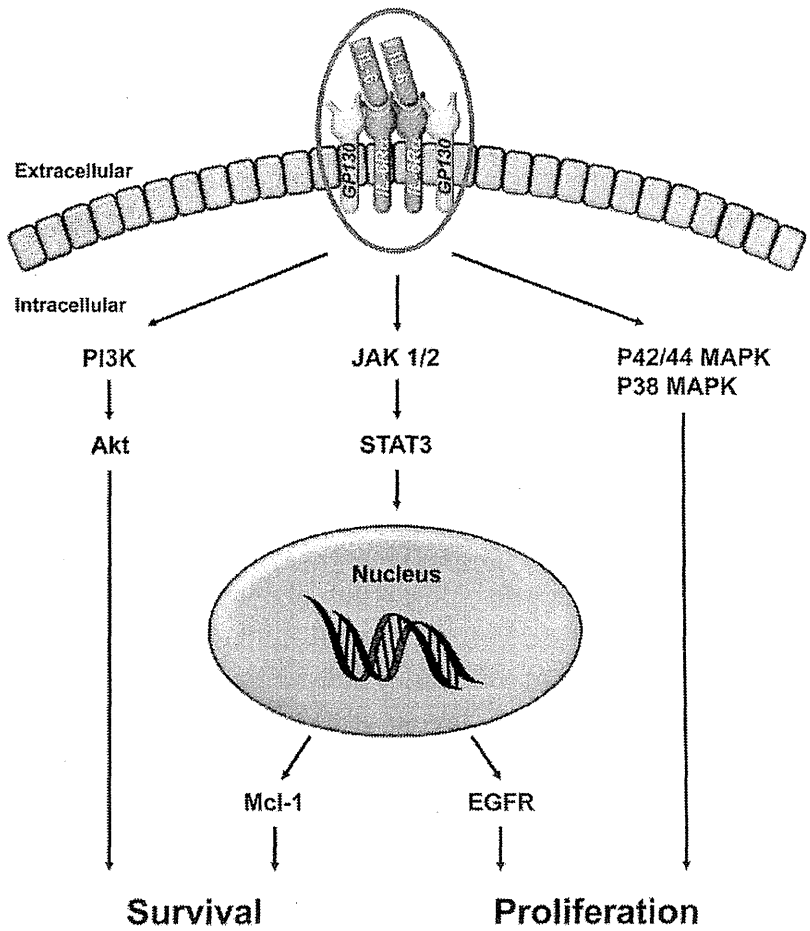


Figure 2. A schematic overview of IL-6 signaling and its downstream effectors in biliary tract cancer.

## ANIMAL MODELS OF GALLBLADDER CANCER

Spontaneous gallbladder cancer is rare in mice and rats.Several papers of chemically induced neoplastic lesions of the mouse gallbladder have been reported [90,91], but a low incidence of gallbladder adenocarcinomas can be induced by continuous administration of either *N,n*-propyl-*N*-formylhydrazine [90] or 2-acetamido-fluorene [91]. In contrast, Hoch-Legeti et al.[92] reported a high incidence of spontaneous gallbladder adenocarcinoma in two inbred strains of guinea pigs. With respect to the cholangiocarcinoma model, the furan rat model described by Sirica and co-workers [93,94] gives rise to a very high incidence of cholangiocarcinoma in the liver. In addition, in the Syrian golden hamster combined treatment with dihydroxy-di-*n*-propyl nitrosamine and liver fluke infestation was associated with enhancement of cholangiocarcinomas and pre-neoplastic lesions in the gallbladder [95].

A group of molecules called receptor tyrosine kinases are closely involved in the signal transduction system related to cell proliferation and apoptosis. ErbB2 is a representative of receptor tyrosine kinase molecules and one of the molecules serving as the origin of this signal transduction system. ErbB2 is largely produced and activated in many cancer cells such as cells of breast cancer, lung cancer, and uterine cancer. Abnormal expression or activation of erbB2 is noted in 30–50% of patients with gallbladder cancer in Japan (higher percentages in the USA and Chile), although the percentage varies across different reports.

Transgenic mice overexpressing erbB2 (BK5.erbB2 transgenic mice), created at the MD Anderson Cancer Center, University of Texas, USA, are destined to develop gallbladder cancer (resembling that in humans) as well as common bile duct cancer and intrahepatic bile duct cancer [96,97]. In this mouse, expression of rat erbB2 cDNA is targeted to the basal layer of multiple epithelial tissues, including the biliarytract epithelia, by a bovine keratin 5 promoter. This mouse line represents the first genetically engineered mouse model for biliary tract cancer. The mechanism for carcinogenesis in this mouse model is considered to involve excessive phosphorylation of the EGF receptor/*erbB2* gene, formation of complexes from EGF receptor/erbB2 gene products, and growth promotion of the biliary tract epithelium due to signal transduction associated with these events.

Some tumorous lesions were found in the gallbladder of about 90% of these mice at the age of 12 weeks. The lesions were histologically rated as hyperplastic epithelium or adenocarcinoma. Analysis of the transgenic mice diagnosed as adenocarcinoma of the gallbladder revealed that gallbladder cancer evolved by two distinct etiological pathways, carcinoma arising from hyperplasia in focal lesion (type HIF Ca, 41%) or hyperplasia in whole epithelium (type HIW Ca, 59%) [98]. Type HIW Ca had a higher frequency of invasiveness compared with that of type HIF Ca. The results suggest that gallbladder carcinoma may arise via the two distinct pathways in the transgenic mouse, and that these pathways are reminiscent of the adenoma-carcinoma sequence and dysplasia-carcinoma sequence observed in human gallbladder cancer.

In the immunohistochemical analysis, fluorescent immunostaining for erbB2 revealed a tendency of fluorescence intensity to increase in the ascending order of normal mouse < hyperplastic epithelium < adenocarcinoma. Phosphorylated erbB2 (p-erbB2; the active form of erbB2) was detected in the areas of hyperplasia and in both type HIF Ca and type HIW Ca of the gallbladder. Strong expression of p-erbB2 was also observed in the areas of invasion. When the proteins extracted from these tissues were analyzed by western blotting, normal mice showed hardly any expression as they did in immunofluorescent staining, whereas the intensity of the bands increased in the order of hyperplastic epithelium < adenocarcinoma. Slight phosphorylation of erbB2 was noted in the hyperplastic epithelium and was more intense in adenocarcinoma. When gefitinib or GW2974 (an inhibitor of EGF receptor and erbB2 tyrosine kinase) was administered to these mice, the level of phosphorylated erbB2 and COX-2 expression decreased, accompanied by reduced cell proliferating potential and enhanced apoptosis, leading to a significant reduction in the incidence of cancer [99]. These experimental results suggest that activation of erbB2 and its downstream signaling pathways is needed for not only gallbladder carcinogenesis but also tumor progression.

## MOLECULES POSSIBLY SERVING AS TARGETS FOR THE TREATMENT OF GALLBLADDER CANCER

Detailed analyses were conducted for the cancer specimens from these mice and clinical specimens from patients with gallbladder cancer and bile duct cancer, focusing on surface molecules (primarily growth factor receptors and cytokine receptors), signal molecules, and infiltration/metastasis factors, to identify tumor biological factors expressed in biliary tract cancer including gallbladder cancer. These analyses yielded the findings on the molecular

mechanisms of onset and progression of biliary tract cancer. Especially, EGFR and/or erbB2, etc. are expected to be useful targets for the treatment of biliary tract cancer. Understanding the growth factor-signaling pathways up-regulated in biliary tract cancer will provide critical clues for novel therapeutic and chemopreventive strategies using drugs and/or agents that selectively target these specific pathways. This part of the paper deals with tumor biological molecules involved in the onset and progression of biliary tract cancer.

## (1) EGF Receptor and erbB2

In connection with the findings from previous studies on BK5.erbB2 transgenic mice, many reports have been published concerning the expression of the EGF receptor or *erbB2* gene in human biliary tract cancer. Among the studies reported from Japan, Nonomura et al. analyzed the frequency of EGF receptor expression (59.5%) in patients with intrahepatic bile duct cancer, stating that the frequency was significantly higher than that in normal controls [100]. Nakazawa et al. analyzed the frequency of *erbB2* gene expression in biliary tract cancer, reporting differences in the frequency depending on its location (15.7% in gallbladder cancer, 11.5% in papillary cancer, 5.1% in extrahepatic bile duct cancer, and 0% in intrahepatic bile duct cancer) [101]. Kawamoto et al. analyzed the frequency of EGF receptor and *erbB2* gene expression in biliary tract cancer, emphasizing the high frequency of *erbB2* gene expression (EGF receptor: 11.7%, phosphorylated EGF receptor: 6.8%, *erbB2* gene: 31.6%, phosphorylated *erbB2* gene: 21.1%) [102]. Thus, EGFR and *erbB2* genes are suitable therapeutic targets for biliary tract and gallbladder cancers, and molecular-targeted treatment directed at the EGF receptor or erbB2 has been shown to be effective in clinical studies, as described later. In addition, it has recently been reported that targeting EGFR/erbB2 pathways enhances the anti-proliferative effect of chemotherapeutic drugs in biliary tract cancers [103].

## (2) Cyclooxygenase-2 (COX-2)

Activation of the arachidonic acid metabolism is important for the onset and progression of inflammation closely involved in the mechanisms of onset or progression of biliary tract cancer; abnormal expression of receptors for a series of enzymes and prostanoids in cancer tissue has been shown to serve as an underlying mechanism of such activation [102–105]. In addition toCOX-2 being important in the arachidonic acid cascade because it is involved in the formation of prostaglandins from the substrate (arachidonic acid), the profiles of the expression of prostanoid receptor subtype (EP) are also important. In this context, it is interesting that some investigators reported mutual regulation between erbB2 oncogene products and COX-2 [106,107].

Regarding the expression of COX-2 in gallbladder epithelium, high COX-2 expression was not found in the normal epithelium, whereas the frequency of high COX-2 expression in the gallbladder cancer epithelium was higher at advanced stages of the cancer (stage II–IV) than that at the early stage (stage I). Interestingly, high COX-2 expression in the stroma was frequently noted in advanced cancer (stage II–IV) but less frequently in the normal epithelium or noncancerous epithelium. COX-2 protein expression level in the gallbladder cancer tissues increases in proportion to the stage of the disease, suggesting that an increase in COX-2 and

its expression in cancer stroma are important factors in determining the progression of gallbladder cancer.

Regarding PGE2, which is formed following excessive expression of COX-2, analysis regarding the signal transduction pathways related to cancer progression is important for the pathophysiological understanding as well as identification of targets for treatment. In gallbladder cancer tissues, mRNA expression was noted for EP2, EP3, and EP4, but not for EP1 [105]. With regard to the distribution of EP2, EP3, and EP4 in tissues, *in situ* hybridization analysis revealed that mRNA signals were consistent with the distribution of glandular epithelium of the cancer-affected area [108]. COX-2, EP2, EP3, and EP4 seem to be important molecular targets for the treatment of biliary tract cancer. In a recently conducted analysis of the effects of various COX-2 inhibitors in animal models of cancer, all COX-2 inhibitors tested were reported to be promising means of treating and preventing biliary tract cancer [109].

## (3) Vascular Endothelial Growth Factor (VEGF)

VEGF is a glycoprotein playing a central role in vascularization within cancer tissue. The density of blood vessels in biliary tract cancer has been reported to serve as a predictor of poor prognosis [110]. The expression of VEGF in biliary tract cancer has been reported to possibly serve as a factor predicting poor prognosis [111,112]. In view of these findings, VEGF is a promising molecular target for the treatment of biliary tract cancer. Bevacizumab is a molecular-targeted drug whose target is VEGF, and clinical trials of this drug in cases of biliary tract cancer are now in progress.

## (4) Hepatocyte Growth Factor (HGF)/c-Met

HGF is a cytokine serving as a powerful growth factor for the normal bile duct epithelium [74]. HGF has been reported to function also as a growth factor in bile duct cancer cells, and cancer cells are known to produce HGF and thus activate cell proliferation [113–115]. The activity of HGF is transmitted via its receptor c-Met; i.e., the binding of HGF to c-Met leads to c-Met dimer formation, self-phosphorylation, and binding to intracellular effectors, resulting in the activation of the downstream pathways [Raf-Ras-mitogen activated protein kinase (MAPK) pathway, phosphatidylinositol-3-kinase (PI3K)-Akt pathway, etc.]. As a result, processesincluding proliferation, migration, and morphogenesis of cancer cells are stimulated. Excessive c-Met expressions are found in more than half of all cases with biliary tract cancer [116,117]. Taken together, these findings indicate that HGF/c-Met is a promising molecular target. At present, drugs such as monoclonal antibodies to HGF and c-Met tyrosine kinase inhibitors are under clinical development.

## (5) TGF-β/IL-6

TGF-β remarkably suppresses the proliferation of normal bile duct epithelial cells. In many malignant tumors, including biliary tract cancer, at least one step of the TGF-β signal transduction pathways is abnormal, leading to TGF-β-induced stimulation of cancer cell proliferation [118,119]. A detailed analysis in an *in vitro* experiment revealed that biliary tract cancer cells produce TGF-β and stimulate themselves through an autocrine or paracrine mechanism, resulting in the formation of the growth factor IL-6 [120]. The disappearance of the effect of TGF-β in stimulating the proliferation of biliary tract cancer cells following siRNA-induced suppression of IL-6 signals allows the assumption that IL-6 is closely involved in the stimulation of cancer cell proliferation by TGF-β. On the basis of these findings, RNA interference (RNAi) therapy has been devised using TGF-β and its intracellular signals (factors that seem to be closely involved in the stimulation of biliary tract cancer growth) as molecular targets and aiming at the suppression of TGF-β receptor II. This therapy is now being evaluated in *in vitro* experiments.

## (6) Mammalian Target of Rapamycin (mTOR)

ThemTOR is a serine/threonine kinase identified as a target molecule for rapamycin (a macrolide antibiotic). This molecule plays multiple roles in the regulation of cell division, growth, and survival. Analysis of the above-mentioned BK5.erbB2 transgenic mice and biliary tract cancer cells suggested that during cellular signal transduction through the erbB family, the erbB2-erbB3 system is primarily involved in the PI3K-Akt pathway, and that the EGFR-erbB2 system is primarily involved in the Raf-Ras-MAPK pathway. Because mTOR is a signaling molecule of the PI3K-Akt pathway, mTOR inhibitors are expected to be useful in suppressing the PI3K-Akt pathway involved in anti-apoptotic activity. Although no report on the clinical application of mTOR inhibitors in the cases of biliary tract cancer has been published, their anti-tumor efficacy has been shown and evaluated in BK5.erbB2 transgenic mice [121].

## (7) Interleukin-4 (IL-4) Receptor

In human intrahepatic bile duct cancer, the frequency of IL-4 receptor expression is higher than normal [122]. *Pseudomonas* exotoxin (PE) is an exotoxin derived from *Pseudomonas aeruginosa* and IL-4 receptor-targeted cytotoxin (IL-4-PE), composed of PE and IL-4, has been shown to efficiently exert cytocidal and antitumor actions against tumors with high IL-4 receptor expression [123,124]. In an experiment on IL-4-PE-induced cell damage, protein synthesis in biliary cancer cell lines was substantially suppressed. When its anti-tumor efficacy was evaluated in nude mice subcutaneously implanted with a tumor, the tumor size decreased significantly in the IL-4-PE-treated groups (administration of IL-4-PE into the tumor or intraperitoneal administration), and the survival period of mice with intraperitoneal dissemination of the tumor was significantly extended following intraperitoneal administration of IL-4-PE [122]. IL-4 receptor cytotoxin therapy is thus

expected to be useful as a new means of molecular-targeted therapy for intrahepatic bile duct cancer.

## (8) Insulin-like growth Factor (IGF) Receptor

The insulin-like growth factor (IGF) receptor and its downstream cellular signals play important roles in the proliferation, apoptosis, migration, and differentiation of cells [125]. Activation of IGF receptor arises from its association with the EGF receptor or from IGF-1 and IGF-2. In cancer cell biology, activation of the IGF receptor and downstream cellular signals are known to be involved in mobility, adhesiveness, and vascularization potentials [126,127]. The frequency of IGF receptor expression in biliary tract cancer is reported to be high [128,129]. In our analysis (data not shown), the expression of IGF receptors did not correlate with any clinicopathological indicators, but the prognosis (survival) was significantly poorer in IGF receptor-expressing cases. The IGF receptor is expected to be useful as a target molecule for molecular-targeted therapy for biliary tract cancer.

## (9) Mucin Core Polypeptide 4 (MUC4)

Several studies have recently shown the involvement of membrane mucins such as Muc1 and Muc4 in cell signaling [77,130,131]. Muc4contains two epidermal growth factor (EGF) domains with conserved amino acid residues of active EGF-like growth factors, one of which reportedly acts as a ligand for erbB2 [77]. Thus, Muc4 acts as a novel transmembrane ligand for the tyrosine kinase erbB2, triggering specific phosphorylation of erbB2 [132]. The expression of MUC4 has been reported in human biliary tract carcinomas [133,134]. In terms of pathobiology for biliary carcinogenesis, these findings can be combined with *erbB2* amplification and/or overexpression in biliary tract carcinomas [71].

The expression levels of MUC4 protein and mRNA were increased in specimens of gallbladder carcinoma. Immunoprecipitation experiments showed an interaction between MUC4 and erbB2. This interaction was associated with the hyperphosphorylation of erbB2, MAPK and Akt and with the overexpression of cyclooxygenase-2. Transfection experiments showed that MUC4 amplifies cell proliferation in the presence of heregulin through potentiating phosphorylation of erbB2 and its downstream signaling pathways. MUC4 is up-regulated and interacts with erbB2 in human gallbladder carcinoma and thereby supports the potential implication of MUC4 in erbB2 activation. Therefore, MUC4 may be a molecular target for a novel therapeutic and chemopreventive approach against gallbladder cancer.

## (10) *N*-Acetylglucosaminyltransferase

It is a well-known fact that oligosaccharide structures are dramatically changed in carcinogenesis including malignant transformation. Oligosaccharides are synthesized by a set of several glycosyltransferases, whose genes are approximately 1% of the human genome. *N*-Acetylglucosaminyltransferase V (GnT-V) is one of the most important among several kinds of glycosyltransferases, which are enzymes involved in carcinogenesis and tumor metastasis

[135-137]. GnT-V is involved in the synthesis of □1-6 GlcNAc branching formation on *N*-glycans. A study of GnT-V-deficient mice clearly showed that GnT-V was essential for tumor growth and metastasis [138]. The mechanisms underlying how GnT-V regulates tumor metastasis involve the up-regulation of signaling of many growth factor receptors on the cell surface by suppressing their endocytosis [139], the enhancement of certain kinds of protease activity [140], and the stimulation of angiogenesis as a co-factor [141].

The expression of GnT-V at the protein level is up-regulated in gallbladder carcinoma tissues and the immunohistochemical expression level of GnT-V is correlated with aggressiveness of the disease, such as the tendency to form distant recurrences, and with postsurgical survival [142]. Moreover, in the *in vitro* and *in vivo* experiments using the gallbladder carcinoma cells, the expression levels of GnT-V in the cells were positively correlated with malignant behaviors, such as rapid cell growth, potent angiogenic capability, and potent metastatic potential. Taken together, the expression levels of GnT-V may serve as a unique biological feature associated with the malignant behavior of gallbladder carcinoma. Therefore, the malignant phenotype can be blocked by a GnT-V inhibitor, Swainsonine, a potent GnT-V inhibitor that reduces tumor metastasis and tumor solid growth in mice [143,144].

## RESULTS OF TREATMENT WITH MOLECULAR-TARGETED DRUGS

Of the target molecules for the treatment of biliary tract cancer discussed in the preceding sections, the EGF receptor, *erbB2* gene, and VEGF, etc., were used as targets in clinical studies designed to evaluate their efficacy.

Erlotinib inhibits the tyrosine kinase domain of the EGF receptor of erbB family members. When used for primary treatment of pancreatic cancer, this drug, in combination with gemcitabine, prolonged the survival period of patients compared to gemcitabine treatment alone. This drug is thus considered as a promising molecular-targeted drug. As far as treatment of biliary tract cancer is concerned, results of a phase II clinical study have been reported on erlotinib monotherapy as a means of initial and secondary treatment [145]. The response rate was low (8%), but the percentage of cases that remained free of aggravation for 24 weeks or longer (the primary endpoint) was relatively higher (17%). However, the prognosis after this therapy did not differ depending on the presence/absence of EGF receptor expression or the intensity of EGF receptor expression.

Lapatinib is a tyrosine kinase inhibitor capable of blocking the tyrosine kinases domains of the EGF receptor and erbB2 among the erbB family members (Figure 7). It is a low-molecular-weight drug designed for oral use. The results of a phase II clinical study on lapatinib monotherapy in patients with advanced biliary tract cancer or advanced hepatocellular carcinoma have been reported [146]. The evaluation of its efficacy revealed that the response rate was 55% for hepatocellular carcinoma, but there was no responder among the cases of biliary tract cancer. Thus, the results were unsatisfactory.

In patients with gallbladder cancer or bile duct cancer having failed to respond to GEMOX therapy (gemcitabine + oxaliplatin), treatment combining these two drugs with cetuximab (a monoclonal antibody to EGF receptor) was attempted. The response rate was