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REVIEW

Cholangiocarcinoma: Recent progress. Part 2: Molecular pathology and treatment

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Abstract Part 2 of this review discusses DNA damage in biliary epithelial cells in the development of cholangiocarcinoma, alterations in cell kinetics of biliary epithelial cells, biliary epithelial mitoinhibition, and apoptosis that includes the role of *Bcl-2*, transforming growth factor-β, telomerase activities and deregulation of *Ras* and *p53*, cancer-associated antigens in cholangiocarcinoma, precancerous lesions, stroma formation and angiogenesis, cancer invasion, cell–cell and cell–matrix interactions, and the mechanism of evasion from immune surveillance. These discussions are followed briefly by treatments such as photodynamic therapy, and surgical approaches comparing resection and liver transplantation.

Key words: angiogenesis, biliary epithelium, dysplasia, *p53*, peribiliary gland, precancerous lesion, stroma formation, transforming growth factor-β.

CELLULAR AND MOLECULAR BIOLOGY OF CHOLANGIOCARCINOMA

There is increasing evidence that the neoplastic transformation of biliary epithelial cells (BEC) and malignant progression of cholangiocarcinomas (CC) is accompanied by a number of molecular and genetic alterations.[1,2] Such cellular alterations at least partly relate to the background hepatobiliary lesions, and the stages or progression of CC.

DNA DAMAGE IN BILIARY EPITHELIAL CELLS IN THE DEVELOPMENT OF CHOLANGIOCARCINOMAS

Genotoxic events usually result in either deoxyribonucleic acid (DNA) repair or, if the damage is beyond repair, deletion of the cells by apoptosis. This may be true in non-neoplastic BEC. However, in CC cells, increased *Bcl-2* expression, *K-ras* mutation, and/or *p53* dysregulation, may inhibit apoptosis after the non-reparable genotoxic event, followed by survival of the mutated cell. Further mutations may lead to malignant transformation and progression through a multistep process.[3,4]

Chronic biliary diseases that predispose to CC, such as hepatolithiasis (Fig. 1) and liver fluke infestation with *Opisthorchis viverrini*, present with the common features of chronic inflammation, cholestasis, and increased BEC turnover. These may be followed by malignant transformation of BEC and progression of CC.[2,5] Inducible nitric oxide synthase (iNOS) expression with nitric oxide (NO) generation is known in several human malignancies in which chronic inflammation is a predisposing factor.[5,6] In chronic biliary diseases, NO and associated reactive oxygen species such as peroxynitrite are produced within and around the inflamed bile ducts. Chronic inflammation with production of inflammatory cytokines, such as interleukin (IL)-1, interferon-γ and tumor necrosis factor-α, by tumor-associated inflammatory cells,[7] and by BEC themselves, induces iNOS, and then sufficient NO and reactive oxygen species in BEC.[2] They may modify or alter DNA bases of BEC and result in direct DNA damage.

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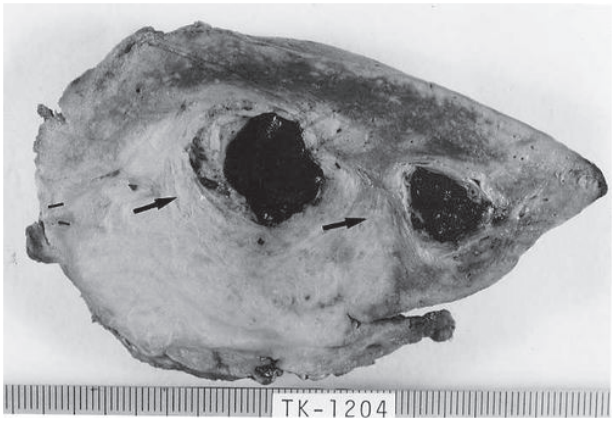

Figure 1 Intrahepatic cholangiocarcinoma of a mass-forming type associated with hepatolithiasis. (→) Intrahepatic calculi.

In addition, DNA repair proteins are potentially susceptible to NO and reactive oxygen species, and this provides another link between inflammation and initiation, and promotion and/or progression of CC in chronic inflammatory biliary diseases.[2] Stimulated NO generation is associated with impaired global DNA repair activity in CC cell lines and genomic instability. Concomitantly, nitrosylation of key repair proteins inhibits the repair of altered DNA followed by the accumulation of potential oncogenic mutations. Inhibition of proapoptotic effector proteins by protein nitrosylation, such as caspase proteases, may also promote extended survival of malignant cells.[8] Induced nitrosylation of caspases would disable apoptotic pathways promoting cell survival. Biliary epithelial cells synthesize reduced glutathione (GSH) by themselves, and they are also able to take it up from the bile secreted by hepatocytes.[9] Reduced glutathione provides the principal intracellular defense against oxidative stress, maintains proteins and other molecules in the reduced state, and participates in detoxification of many molecules in BEC.[10] In experimentally induced cholestasis, GSH is decreased in bile. The enzymes indispensable for the GSH synthesis are also reduced or lost in damaged BEC in patients with chronic biliary disorders. Such alteration of GSH content in BEC may deregulate apoptosis, and also lead, indirectly, to DNA damage.

ALTERATIONS IN CELL KINETICS AND MOLECULAR MECHANISMS

Biliary epithelial proliferation

Several cytokines or growth factors are known to have mitogenic or proliferative effects on BEC, such as IL-6, hepatocyte growth factor (HGF), transforming growth factor (TGF)-α, epidermal growth factor (EGF), *c-erbB-2*, heterogeneous immunoglubulin A (IgA), and leukocyte inhibitory factor (LIF) via an autocrine or paracrine effect.[11–14] Their receptors are constitutively expressed or secondarily induced to be expressed on BEC. For example, LIF receptor, IL-6 receptor (IL-6R) and EGF-receptor (EGFR) are expressed on BEC.[12] *Met*, a receptor of HGF, and *c-erbB-2*, which encodes a transmembrane protein highly homologous to EGFR, are pathologically induced and expressed on BEC.

Non-cancerous biliary epithelium

During reactive proliferation, competing mitogenic and mitoinhibitory influences acting on BEC are accompanied by up-regulation of HGF and IL-6 in peribiliary stromal and hematolymphoid cells.[7–10,13–15] Their receptors, *met* and IL-6R protein, are also up-regulated in BEC. Interleukin-6 is able to directly stimulate DNA synthesis and inhibit apoptosis of BEC, via an increase of the *Bcle-2/bax* ratio.[16] Non-neoplastic BEC that are either stressed, injured or cultured can also produce and secrete IL-6, which can then act as an autocrine growth factor.[12] Normal human BEC show weak or no expression of IL-6 mribonucleic acid (RNA) and protein, but IL-6 mRNA and protein are up-regulated in damaged or stressed BEC.[6,14] In contrast, HGF/*met* signalings must be based on a paracrine effect. *Met* was in fact frequently over-expressed in proliferative BEC,[16] suggesting that HGF/*met* may play an important role in biliary hyperplasia. In addition, TGF-α and its receptor (EGFR) are expressed in interlobular bile ducts, proliferating bile ductules, and most hepatocytes in various hepatobiliary liver tissues.[12] In hepatolithiasis, there is frequent overexpression of *c-erbB-2*[11] in the proliferated intrahepatic bile ducts and proliferated peribiliary glands.

Cholangiocarcinomas

Cell kinetic studies disclosed that the proliferative activities of BEC increase with the histologic progression from biliary epithelial hyperplasia through biliary dysplasia to carcinoma *in situ* and invasive intrahepatic CC.[17] Interleukin-6 and HGF are potentially mitogenic, and neoplastic transformation of BEC is associated with constitutive production of IL-6 and HGF with acquisition of IL-6/*gp130* and HGF/*met*-based autocrine and/or paracrine control circuits *in vitro* and probably *in vivo*.[18,19] Mitogen-activated protein kinase (MAPK) cascade appears to be a key signaling pathway in the regulation of non-neoplastic and neoplastic BEC growth via HGF/*met* and IL-6/*gp130* signalings.[18] Hepatocyte growth factor, its mRNA and *met* are expressed aberrantly in CC cells, and in putative precancerous intestinal metaplastic epithelium induced in the liver of furan-treated rats, suggesting that the HGF/*met* autocrine loop is established as a critical event in this animal model.[20] Cultured CC cells continue to grow in basal serum-free medium and spontaneously produce HGF, resulting in auto-phosphorylation of *met*, indicative of autocrine growth control circuits by using HGF/*met* in CC.

Interleukin-6 and its mRNA are detectable in intrahepatic CC cells *in vivo* and also in CC cell-lines.[19] Stimulation with IL-6 results in proliferation of intra-

hepatic CC cell-lines, which possess the IL-6 receptor complex subunits, suggesting that IL-6 works with receptor-mediated signalings. In fact, *in vitro*, both cultured BEC and CC lines express *met* and *gp130* mRNA and protein, but the levels of expression are higher in the latter. In both, exogenous HGF or IL-6 induces phosphorylation of *met* or *gp130*, respectively, with a concentration-dependent increase in DNA synthesis. Interleukin-6 protein/mRNA production and increased serum IL-6 levels can be detected in 90% of intrahepatic CC patients.[19] Spontaneous IL-6 production in intrahepatic CC could be caused by constitutive activation of promoters and/or defects in tumor suppressor genes. Both *p53* and retinoblastoma constructs have binding sites on an IL-6 repressor element, suggesting that inactivation of these tumor suppressor genes may lead to enhanced IL-6 synthesis and then activity. Aberrant expression of *c-erbB-2* is found frequently in CC, suggesting that the *c-erbB-2* oncogene participates in cholangiocarcinogenesis, and this may be used as a phenotypic marker for neoplastic transformation of BEC.[11]

Biliary epithelial mitoinhibition and apoptosis

Neoplastic transformation and malignant progression of BEC may be a result, in part, of failure in activating apoptosis and deleting cells with genetic damages.[17,21]

Bcl-2

Bcl-2 protein is expressed by bile ductules and interlobular bile ducts, but not by the large intrahepatic bile duct, while *Bax*, a promoter of apoptosis, is expressed along the whole biliary tree.[22] Homeostasis of BEC through a balance between apoptotic cell death and cell renewal, is mainly regulated by these *Bcl-2* family proteins.[16] Resistance to apoptosis, by altered expression of *Bcl-2* family members, has been implicated as a mechanism contributing to malignant transformation. Overexpression of *Bcl-2* may contribute to neoplastic expansion by prolonging cell survival through *Bcl-2* suppression of the physiological cell turnover mechanisms. Immunocytochemistry has indicated that the *Bcl-2* gene is generally expressed in bile duct cells and also in CC.[23] The rate of *Bcl-2* overexpression is variably reported, probably because of the methods used, and also to the difference of intrahepatic CC specimens in the liver,[24,25] although high contents of *Bcl-2* mRNA were found also in most CC cases. Intrahepatic CC cells were resistant to apoptosis because of beauvericin, which is known to induce apoptosis of non-neoplastic BEC in a concentration- and time-dependent manner. *Bcl-2* protein expression was 15-fold greater in malignant than in non-malignant BEC. Antisense oligonucleotide reduced expression of *Bcl-2* protein by 50% and increased the rate of beauvericin-induced apoptosis more than threefold in CC cells. Deregulation of *Bcl-2* expression in CC is inactivation of the tumor suppressor gene, *p53*, which is known to suppress *BC-2* expression (see below). In CC, the expression of *Bcl-2* was inversely related to lymph node metastasis, vascular invasion, perineural invasion, *Ki-67* labeling index, aberrant *p53* expression and incidence of apoptotic cells.

Transforming growth factor-β

Many malignant tumors harbor defects in TGF-β signaling and are resistant to TGF-β-mediated growth suppression. So far, a close correlation between disruption of the TGF-signaling pathway and deregulated growth of cancer cells has been demonstrated in malignant tumors, including biliary tract carcinoma. Transforming growth factor signaling pathway disturbance could be a result of genetic changes in the TGF-β type I and II receptor gene, followed by genetic inactivation of TGF-β in biliary cancers.[26,27] In fact, homozygous or heterozygous deletion of the TGF-β type I receptor gene is identified in biliary adenocarcinomas. Inhibition of DNA synthesis in CC cells via up-regulation of *p21/Wafl* expression caused by TGF-βl could be another mechanism operating to cause the defect in TGF-β, because HuCCT human CC cells present such data,[28] although other CC cell lines are resistant to TGF-mediated growth inhibition.[18]

Telomerase activities

Human telomerase RNA and telomerase-associated protein-1 mRNA are demonstrable in biliary dysplasia and in intrahepatic CC, irrespective of histology.[29] The signals are homogeneously detected in intrahepatic CC (Fig. 2), while their distribution was more or less heterogeneous and focal in dysplastic foci. These signals are not detected in non-dysplastic biliary epithelia in hepatolithiasis and normal livers, suggesting that CC acquires telomerase activities at an early development stage of intrahepatic CC (biliary epithelial dysplasia), and that this immortalization may be involved in the further malignant progression.

Deregulation of *ras* and *p53*

K-ras

Activation of *K-ras* oncogenes by point mutation is detected in CC.[30] By sequencing, *K-ras* point mutation in CC has been found in codon 12 with changes from glycine (GGT) to aspartic acid (GAT) or less often to valine (TGT). Less frequent mutations have been seen at codon 13, involving GGT to GAT, and codon 61, involving CAA to CAC.[31–33] The incidence of *K-ras* mutations is 100% in English[34] and 4–60% among Japanese and Thai patients.[1,35,36] The variable incidence of *K-ras* mutations may reflect the location of CC, because *K-ras* is crucial for carcinogenesis originating downstream of the biliary tree. In fact, *K-ras* point mutation is frequent in the periductal-spreading type but absent in the mass-forming type, suggesting that *K-*

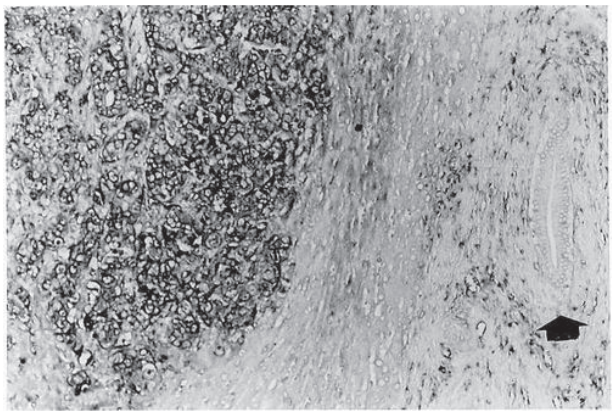

Figure 2 Intrahepatic cholangiocarcinoma (left half) shows marked and diffuse expression of human telomerase RNA (hTR). Non-neoplastic bile duct (→) is negative for hTR. *In situ* hybridization with antisense probe for hTR.

ras mutation is important in CC arising near the hepatic hilus.[3,4,37] These different rates may also reflect different etiologies; the incidence ranges from 58% in sporadic CC to 8% in CC related to *O. viverrini* in Thailand.[5]

p53

Impairment of the *p53* pathway is an important step in cholangiocarcinogenesis. Dysfunction of this gene either by mutation or *mdm-2* gene amplification is observed in one-half of intrahepatic CC cases. *p53* has two functions, including initiation of cell cycle arrest and suppression of *bcl-2* expression. In fact, *p53* protein accumulated and was immunohistochemically detectable in 25–75% in intrahepatic CC,[1] particularly in the well- and moderately differentiated CC. *p53* gene mutations have been detected in roughly 5–35% of intrahepatic CC,[1,3] particularly in the mass-forming type, suggesting that *p53* mutation is related to the development of intrahepatic CC arising in the peripheral small bile ducts. The most common mutation of the *p53* gene is A–G transition, mostly missense mutations and less frequently nonsense mutations.[3] There may be some epigenetic phenomena that stabilize *p53* protein in CC. That is, wild type *p53* may be stabilized and then become detectable by forming complexes with other molecules of *p53* downstream effector genes, such as *WAF-1* and *mdm-2*.[1] Both proteins are frequently detected in the cases positive for *p53* protein. The association of *mdm-2* oncoprotein to *p53* protein is also known to be one of the mechanisms of *p53* inactivation.[1]

Cancer-associated antigens in cholangiocarcinomas and precancerous lesions

A number of human mucin antigen genes (*MUC1–9*) have been cloned.[38] Biliary epithelial cells in intrahepatic large bile ducts and peribiliary glands constitutively express *MUC3* and *MUC2*, respectively,[17] while the *MUC* profile is altered in CC.[38] *MUC1* is expressed in a majority of CC, and *MUC2* is also expressed in some CC. Invasive CC with a poor outcome express *MUC1* but are negative for *MUC2*.[38,39] Expression of *MUC1* may be useful as a prognostic indicator of poor patient survival.[39] In contrast, *MUC2* expression is related to a favorable prognosis;[40] *MUC2* expression is relatively frequent in well-differentiated and non-invasive CC, and also biliary cystadenocarcinoma.[17,38] *MUC5AC/6* apomucin is more frequently expressed in well-differentiated CC.[38]

In normal livers, *CK7–9* and *CK19* are expressed on the intrahepatic biliary tree, while *CK8* and *CK18* are expressed on hepatocytes.[40,41] In a majority of CC, particularly well- to moderately differentiated types, *CK7* and *CK19* expressions are frequent and extensive in carcinoma cells. *CK20* expression is focal and infrequent in moderately and poorly differentiated peripheral CC, and frequent but focal in hilar CC.[40,41] This characteristic *CK* profile helps to differentiate between CC and metastatic adenocarcinomas.

STROMA FORMATION IN CHOLANGIOCARCINOMA

Fibrous stroma is a common feature of CC. Non-neoplastic BEC promotes fibrogenesis by a number of mechanisms, including the expression and/or activation of growth factors, such as TGF-β1, platelet-derived growth factor, and endothelin-1. Fibrogenic stromal cells positive for α-smooth muscle actin (ASMA) are divisible into peritumoral perisinusoidal cells and intratumoral stromal cells. Both types of cells are abundant in CC and their numbers show a significant positive correlation with the degree of tumor fibrosis. They produce extracellular matrix (ECM) proteins and other molecules followed by fibrosis.[42] The peritumoral ASMA-positive perisinusoidal cells are frequently in direct continuity with intratumoral ASMA-positive stromal cells in CC, and peritumoral perisinusoidal cells transform into ASMA-positive activated perisinusoidal cells (myofibroblasts); they are incorporated into the tumor as intratumoral ASMA-positive stromal cells.

Heparan sulfate proteoglycan (HSPG), one of the basement membrane constituent macromolecules, is intensely detectable in the rich stroma of intrahepatic CC; it plays important biological roles in cellular growth, differentiation, cell-matrix adhesion, motility, invasion and metastasis of carcinoma cells.[43] Such functions of HSPG are mediated by its large reservoirs of heparin-binding growth factors such as epidermal growth factor (EGF), TGF and HGF in the extracellular milieu outside the basement membrane. Midkine, a novel heparin-binding growth factor with survival-promoting and migration-enhancing activities, is intensely detected in the cytoplasm of intrahepatic CC, particularly the well- and moderately differentiated types.[44]

ANGIOGENESIS OF CHOLANGIOCARCINOMA

Rapid growth of malignant tumors is accompanied by increased angiogenesis. Cholangiocarcinomas and the surrounding tissue may produce various factors that affect angiogenesis; it depends on the balance of angiogenesis activators and inhibitors. Enhanced expression of thrombospondin-1 (TSP-1), an antiangiogenetic factor,[45] was observed in the fibroblasts of tumor stroma, as well as cancer cells, suggesting that TSP-1 in both cancer cells and tumor stroma may negatively regulate angiogenesis in intrahepatic CC.[45] Up-regulation of TSP-1, together with down-regulation of vascular endothelial growth factor in cancer cells, may have a role in the hypovascularity of CC. In addition, enhanced expression of TSP-1 in both tumor and stromal tissue of CC might provide a favorable environment for tumor invasion, by promoting attachment, migration, and invasion.[46] Of the other angiogenetic factors, NO may confer a survival advantage by serving as an angiogenetic agent.[47] The strong reactivity for the components of HSPG, particularly syndecan-3 and perlecan in tumoral stromal vessels, might suggest a role for HSPG in tumoral angiogenesis.

INVASION AND CELL–CELL AND CELL–MATRIX INTERACTIONS

Many proteolytic enzymes, collectively called matrix proteinases, are involved in the invasion of CC by degrading ECM proteins. Matrix metalloproteinases (MMP), pancreatic trypsinogen and cathepsin B, and urokinase-type plasminogen activator play such roles.[46,48] In intrahepatic CC, MMP-1, MMP-2, MMP3, tissue inhibitor of MMP (TIMP)-1, and TIMP-2 are frequently expressed in tumor cells and/or tumor stroma.[48] The expression of MMP and TIMP was located in the cytoplasm of carcinoma cells with a diffuse or granular pattern, and that in the tumor stroma was seen in fibroblasts, leukocytes, and ECM. Their expression was strong in intrahepatic CC, with severe invasion compared to that with mild invasiveness. The CC stroma positive for pancreatic trypsinogen frequently shows destructive features. These findings suggest that intrahepatic biliary cells may neoexpress or overexpress MMP and TIMP, as well as pancreatic trypsinogen and cathepsin B after malignant transformation, and that these matrix proteinases work during invasion by degrading ECM. Urokinase-type plasminogen activator receptor is frequently expressed, mostly by host cells distributed along the tumor–host interface.

CD44 is a family of transmembrane glycoproteins that act mainly as a receptor for hyaluronan. It can also bind to some other ECM ligands with lower affinity. *CD44* is composed of the standard, ubiquitously expressed isoform (*CD44s*), and variant isoforms (*CD44v*). *CD44s*, *CD44v5*, *CD44v6*, *CD44v7–8*, and *CD44v10* are reportedly neoexpressed on carcinoma cells during carcinogenesis of CC, but this neoexpression does not correlate with tumor progression, with the exception of *CD44s* and *CD44v5*. The aberrant expression of *CD44s* significantly correlates with the absence of metastasis and vascular invasion of intrahepatic CC, and *CD44v5* aberrant expression significantly correlated with *p53* under-expression.[49]

Galectins, a family of β-galactoside-binding animal lectins, may be involved in the malignant transformation and progression of CC. Galectin-3 expression is up-regulated in the preneoplastic and early neoplastic stages of intrahepatic CC, although it tends to disappear at a later stage, suggesting that galectin-3 is rather associated with malignant transformation of BEC. This feature is different from other malignancies in which galectin-3 is known to be a critical determinant for anchorage-independent survival of disseminating cancer cells in circulation during metastasis; up-regulation of galectin-3 is also reported to be associated with tumor progression.[50] By contrast, galectin-1 is aberrantly expressed in the carcinoma cells of two-thirds of intrahepatic CC cases, particularly at progressive stages, and this expression seems to be associated with proliferative activities, histologic differentiation, and invasive characters.

EVASION FROM IMMUNE SURVEILLANCE

During carcinogenesis and progression of CC, carcinoma should evade from the host immune surveillance. Several processes for the evasion have been reported. Increased apoptosis of host immunological cells by carcinoma cells is thought to play a key role in this process. Recently, it was shown that a tumor evasion mechanism involving *Fas*/*FasL* exists in intrahepatic CC; frequent and strong expression of *FasL* in biliary dysplasia, and well-differentiated intrahepatic CC enables them to escape from immune surveillance by counter-attacking *Fas*-bearing infiltrating lymphocytes.[51] The finding that infiltrating lymphocytes were more frequently apoptotic within intrahepatic CC foci than in non-neoplastic foci remote from intrahepatic CC foci, also supports this hypothesis. This counter-attack becomes insensitive in moderately and poorly differentiated intrahepatic CCs where the down-regulation of *Fas* gives them a resistance against the *FasL*-expressing lymphocytes.

Galectin-1 and *MUC-1* are also known to induce T-cell apoptosis and protect carcinoma cells from cytotoxic T-cell interaction. Therefore, it seems plausible that accumulated or leaked galectin-1 and *MUC-1* in the stroma around carcinoma cells may act as an immunological shield by inducing activated T cells[52] to form a microenviroment favorable for cell growth and invasion of carcinoma cells, and promote infiltration, metastasis at their boundaries and then tumor progression.[53] Sialic acids of *MUC-1* oligosaccharides on the surfaces of tumor cells[54] may also contribute significantly to the inhibition of cell–cell and cell–substratum interactions.[56]

TREATMENT

The prognosis of CC remains very poor and, despite various new attempts for therapy, no breakthrough has been achieved. Photodynamic therapy is a new non-surgical two-step procedure to destroy cancer exposed to the bile duct lumen,[56–58] but is applicable only to hilar CC, and its efficacy is not yet established.

Hepatic resection offers the only hope for long-term survival of CC patients. The reported 5-year survival in the resected patients varies from 0 to 42%, and the median survival from 8 to 59 months.[59–61] Attempts have recently been made to determine the factors affecting the prognosis after resection. Yamamoto *et al.*[62] morphologically classified intrahepatic CC into mass-forming and infiltrating types: the 5-year survival rate was 44% for the former and 27% for the latter. Advanced hilar carcinoma is difficult to manage, but clearly aggressive resection affords better results.[63] Nagino *et al.* determined the exact extent of invasion along the biliary tract by percutaneous transhepatic biliary drainage, and in patients in whom extensive resection was required, they excluded the portal vein supplying the lobe to be resected, and carried out resection 2 weeks later. During this period, the other lobe enlarged by 10%, preventing postoperative hepatic failure.[64]

Molecular biological parameters have recently been investigated as prognostic markers for intrahepatic CC. It has been suggested that *K-ras* gene alteration is involved in the carcinogenesis of infiltrating type intrahepatic CC[65] and that 50% of cases with infiltrating CC have loss of heterozygosity in chromosome *8p*.[66] However, Tannapfel *et al.*[67] examined the correlation between mutation of the *p53* tumor suppressor gene, the degree of apoptosis, and proliferation by molecular biological approaches, and failed to establish that these parameters predict the prognosis.

Liver transplantation has been performed in selected patients. Cherqui *et al.*[68] advocated aggressive surgical management, including total hepatic resection and liver transplantation, but Berda *et al.*[69] failed to demonstrate a beneficial effect on survivial by total hepatectomy and liver transplantation. The number of patients was small in these series; however, Casavilla *et al.*[70] performed liver transplantation in 12 patients, and hepatic resection in 42 patients with intrahepatic CC over a period of 14 years. In their series, the mortality within 1 month was 7.4%, and the 5-year survival rates after hepatic resection and liver transplantation were 31% and 18%, respectively: the difference between the two treatments being insignificant. They recommend that hepatic resection is as effective as transplantation when intrahepatic CC can be removed with adequate margins, when the lesion is singular, and when lymph nodes are not involved. However, according to Pichlmayer *et al.*[71] and Jeyarazah *et al.*,[72] the outcome after liver transplantation for intrahepatic CC remains poor, even after the use of aggressive adjuvant radiotherapy or cluster transplantation technique, with a 3-year survival of 30% at best. These transplantation results[71,73–75] are much inferior to those reported with hepatocellular carcinoma (HC).[76] One of the reasons is delay in tumor detection; HC patients have chronic liver disease and HC is found in its early stage before spread occurs. Another reason may be early lymphatic transport of cancer cells in CC; lymph node metastasis is rare in HC before the advanced stage, whereas it is very common in CC. Because of the generally poor surgical results, many surgeons have emphasized the necessity of new adjuvant treatment.

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