Chapter 2
Overview of Cholangiocarcinoma and Evidence for a Primary Liver Carcinoma Spectrum

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Abstract Intrahepatic cholangiocarcinoma, second in incidence to hepatocellular carcinoma among the primary liver carcinomas, has an even more dismal prognosis. Intrahepatic cholangiocarcinoma is difficult to diagnose at an early stage of development and advances aggressively, with widespread metastases. Molecular genetic features of intrahepatic cholangiocarcinoma have been partially elucidated, although the specific genetic lesions and molecular processes that drive its development, progression, and metastasis are still obscure. Evidence has accumulated from many sources suggesting that cholangiocarcinoma and hepatocellular carcinoma are components of a spectrum of primary liver carcinomas, including poorly and aberrantly differentiated varieties. Primary liver carcinomas arise from cells in different stages of development that encompass the entire lineage of liver epithelial cells generated from hepatoblasts and/or adult liver stem cells, and share critical genomic aberrations and phenotypes with these progenitor cells.

Keywords Primary liver cancer · Hepatocellular carcinoma · Intrahepatic cholangiocarcinoma · Overlap of primary liver cancers

1 Introduction

The primary tumors of the liver comprise a heterogeneous group of benign and malignant neoplasms that include representatives of each of the cellular elements of which the liver is composed – various epithelial, mesenchymal, and vascular cells and the multicellular structures that are made of combinations of these cells (Grisham 2009). Reflecting the cellular composition of the fully developed liver, the majority of the primary liver tumors are epithelial, the better-differentiated varieties resembling the cytology of either the more numerous hepatocytes of the metabolically complex hepatic parenchyma or the less numerous cholangiocytes of the bile ducts that connect the liver parenchyma to the gut. Clinical and pathological studies

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beginning in the mid-nineteenth century gradually defined the natural histories and the pathological features of the major malignant epithelial tumors of the liver, the primary liver carcinomas (PLC) – hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC).

Well-differentiated HCC contains histological structures that resemble hepatic plates and delicate microvessels with little supporting connective tissue, while well-differentiated ICC is composed of histological structures that mimic bile ducts and the generally dense connective tissue that encloses them (Goodman 2007). Since the better-differentiated PLC reflect cytological and histological features of mature hepatocytes and cholangiocytes, HCC and ICC are often considered to be neoplastic variants of the mature hepatic epithelial cells. However, many PLC are cytologically ambiguous and do not closely resemble either mature hepatocytes or cholangiocytes. Examination of the phenotypic properties of the component epithelial cells of PLC has disclosed that both well-differentiated and poorly differentiated tumors may be composed of cells that reflect mixed properties of both mature hepatocytes and cholangiocytes (Kim et al. 2004). Reflecting the embryonic development of hepatocytes and cholangiocytes (Lemaigre and Zaret 2004; Zaret and Grompe 2008; Zhao and Duncan 2005), current evidence suggests that PLC comprise a continuous spectrum of related malignant neoplasms that includes well-differentiated hepatocellular carcinoma and cholangiocarcinoma that closely mimic morphological and phenotypic properties of either hepatocytes or cholangiocytes, as well as cancers that express mixtures of the phenotypic properties of both mature cell types and of the immature cells that are their precursors.

In this chapter we present a brief overview of intrahepatic cholangiocarcinoma, or ICC, followed by a discussion of the evidence that PLC form a spectrum of closely related tumors derived from common precursors, together with implications of this relationship for further definition of the cellular origins and prognostic categories of individual PLC.

2 Overview of Cholangiocarcinoma

Cholangiocarcinoma or bile duct carcinoma can occur anywhere along the system of bile ducts, from the point at which the terminal (smallest) bile ducts connect to biliary canaliculi located between hepatocytes of hepatic plates, through progressively larger bile ducts within the liver, to a single common bile duct (extrahepatic) which connects intrahepatic ducts with the duodenum. Accompanied by branches of the portal vein and hepatic artery, the common bile duct divides into two branches (right and left hepatic bile ducts) at the entrance into the liver (the liver hilum) (Grisham 2009). Cholangioarcinoma arising in the right and left hepatic ducts at the liver hilum and in the common bile duct and/or ampulla of Vater express growth patterns and genotypic/phenotypic properties that differ from ICC (Henson et al. 1992; Suto et al. 2000), likely reflecting differences in the embryonic origin and development of extrahepatic and intrahepatic bile ducts (Lemaigre

and Zaret 2004; Zaret and Grompe 2008). By convention extrahepatic and intrahepatic cholangiocarcinomas are considered to be separate neoplasms (Nakanuma et al. 2003).

Intrahepatic bile ducts form 8 to 10 branches within the liver that vary in size and structure (Grisham 2009). The largest intrahepatic bile ducts (septal and segmental ducts) contain cholangiocytes that show mucinous and serous differentiation and are associated with peribiliary glands embedded in a dense collagenous stroma. Smaller septal and interlobular branches are composed of a single layer of cuboidal cholangiocytes that lack apparent specific differentiation and are surrounded by a basal membrane and less dense collagenous connective tissue. Septal and interlobular ducts are usually located within portal tracts, where they are surrounded by a rich capillary plexus. The smallest branches, terminal bile ducts (ductules or cholangioles), emerge from portal tracts and extend into the liver parenchyma where they connect with bile canaliculi at the periphery of hepatic plates. The connections between cholangiocytes of terminal bile ductules and hepatocytes of hepatic plates form tubular structures composed of both small cholangiocytes and hepatocytes, called the Canal of Hering (Grisham 2009). The Canals of Hering are thought to be the major intrahepatic sites that enclose adult liver stem cells (Kuwahara et al. 2008).

ICC can arise from any part of the intrahepatic bile ducts (Nakanuma et al. 2003). ICC is less frequent than HCC by a ratio of 1 ICC to about 8–10 HCC (Bosch et al. 2004). However, the incidence of ICC varies geographically and is the most frequent PLC in parts of southern Asia (Bosch et al. 2004). ICC appears to be increasing in incidence world-wide (McGlynn et al. 2006; Schurr et al. 2006; West et al. 2006). In contrast to well-differentiated HCC composed of plates of large, eosinophilic cells that morphologically resemble hepatocytes together with capillary-sized vessels and sparse connective tissue, well-differentiated ICC contains smaller cuboidal or columnar cells with pale eosinophilic or clear cytoplasm that form duct-like structures (adenocarcinoma) enclosed in more-or-less dense arrays of matrix molecules (collagens and components of basal membranes) (Goodman 2007). ICC forms tumors that predominantly grow within the intrahepatic ducts, forms localized scirrhous nodules, or spreads widely through the liver mass (Nakanuma et al. 2003; Goodman 2007).

Clinical experience, confirmed by epidemiological studies, shows that ICC has a worse prognosis than does HCC (Blechacz and Gores 2008). As with HCC, diagnosis of ICC at an early stage of tumor development is difficult, impairing the success of potentially curative therapy. Neoplastic cholangiocytes may express mucin core protein (MUC), carcinoembryonic antigen (CEA), and other cancer-associated proteins, but these tumor-derived molecules have limited specificity and sensitivity as markers of early ICC and lack efficacy for early diagnosis (Blechacz and Gores 2008). ICC usually develops in a noncirrhotic liver, thus, is often advanced in development at the time of diagnosis, and metastasizes widely to organs outside the liver (notably to lymph nodes and bones). Tumor cells of ICC may express a variety of aberrant differentiations, including squamous, clear cell, and sarcomatous. Malignant tumors associated with terminal bile ductules (cholangiolocellular

or ductular carcinomas) often contain mixtures of neoplastic ductular epithelium and hepatocytes (Steiner and Higginson1959; Komuta et al. 2008). Mixed HCC/ICC that contain both neoplastic hepatocytes and cholangiocytes also occur in situations in which an association with terminal bile ductules is uncertain (Allen and Lisa 1949; Goodman et al. 1985).

Major risk factors for the development of ICC have the common feature of producing chronic inflammation in and around bile ducts associated with recurring damage to cholangiocytes, with or without bile stasis. Particular factors long associated with high risk for ICC include chronic infections with the biliary flukes, *Opisthorchis viverrini* and *Clonorchis sinensis*, primary sclerosing cholangitis, hepatolithiasis, and congenital segmental dilation or cysts of bile ducts (Shaib and El-Serag 2004). Liver fluke infestation is particularly important in parts of southern Asia where ICC is a predominant PLC (Vatanasapt et al. 1995).

Chromosomal aberrations are frequent in ICC, with losses involving loci on 1p, 3p, 4q, 8p, 9p, 13q, 16q, and 17p, and gains involving loci on 1q, 3q, 5p, 6p, 7p, 8q, 12q, 15q, 17p, 18p, and 20q in more than 20% of 76 ICC examined by comparative genomic hybridization (CGH) (Koo et al. 2001; Wong et al. 2002; Lee et al. 2004; Uhm et al. 2005). Associated with these locus losses and gains are aberrations in expression of several genes that modulate the metabolism of cholangiocytes and participate in cellular processes that regulate birth, death, vascular supply, and invasion/metastasis of affected tumor cells. Included among these alterations are frequent mutations in *p53*, $p16^{INK4A}$, $p21^{WAF/CIP}$, *DCP4/Smad4*, *TGFβR*, and *Kras* genes (Fava et al. 2007). In many ICC these genetic aberrations are associated with up-regulation and over-expression of several regulatory molecules, including telomerase, Bcl-2, Bcl-X_L, Mdm-2, Mcl-1, IL-6 and IL6R/gp130, HGF/cmet, c-Erb-B2, COX-2, MMP, TGF-β, and VEGF in some combination (Nakanuma et al. 2003; Berthiaume and Wands. 2004; Sirica 2006; Fava et al. 2007). Nevertheless, the particular genetic changes and molecular pathways that drive the development of ICC are not yet understood, and the eradication of tumor development by molecular genetic methods is not yet possible. Most of the studies on which these data are based have examined only one or two genes or molecules in a relatively small number of ICC that likely represent a heterogeneous mixture of tumors representing different stages of development and progression. Only a few studies employing global analysis of gene expression of ICC have been published (Obama et al. 2005; Woo et al. 2009), precluding the precise delineation of the varieties of complex gene signatures that characterize these cancers.

3 Evidence that PLC Comprise a Spectrum of Closely Related Tumors

Mounting evidence indicates that HCC and ICC are components of a PLC tumor spectrum that includes well-differentiated hepatocellular carcinomas and cholangiocarcinomas, which express phenotypic properties that reflect major features of

the complex phenotypes of mature, fully differentiated hepatocytes or cholangiocytes, respectively. Mixed HCC/ICC may contain more-or-less well-differentiated examples of both hepatocytes and cholangiocytes (Allen and Lisa 1949; Goodman et al. 1985). Analysis of genetic aberrations in HCC and ICC components separated from a few mixed HCC/ICC suggests that both types of cells are genomically monoclonal, and arise from a common precursor cell (Imai et al. 1996; Fujii et al. 2000; Murata et al. 2001). Intermediate between the well-differentiated PLC are a variety of incompletely or aberrantly differentiated tumors in which individual tumor cells express different combinations, or mixtures, of the phenotypic properties of mature non-neoplastic hepatocytes and cholangiocytes (Kim et al. 2004), as well as tumors that express aberrant differentiations (squamous, clear cell, sarcomatous, etc.) not ordinarily found in either type of non-neoplastic cell.

The opinion that PLC form a unitary continuum of closely related tumors receives substantial conceptual support from new insights into the embryonic development of the liver, specifically the elucidation of cellular pathways by which differentiated hepatocytes and cholangiocytes are formed from primitive epithelial cells derived form the embryonic foregut. It is now well-established that both hepatocytes and intrahepatic cholangiocytes are derived from a common precursor cell, the hepatoblast, a direct descendant of epithelial cells that migrate from the foregut into the embryonic septum transversum (Zaret and Grompe 2008). Other important support comes from studies on the replacement of hepatocytes and cholangiocytes in livers injured by pathological processes (Fausto and Campbell 2003). Both mature hepatocytes and cholangiocytes can proliferate repeatedly to replace lost or damaged cells when proliferation of the residual mature cells is not impeded. Much evidence now demonstrates the ability of stem cells, located predominantly in the Canals of Hering, to generate new hepatocytes and cholangiocytes even when the residual populations of fully differentiated cells are unable to proliferate. During the process of replacing differentiated hepatocytes and cholangiocytes from stem cells, a transient population of phenotypically heterogeneous intermediate cells, which express various partial combinations of phenotypic properties of the fully differentiated cells and their cellular precursors, is generated (Fausto and Campbell 2003). Phenotypically heterogeneous intermediate cells undergo further differentiation to acquire the specific complex phenotypes that characterize fully differentiated hepatocytes and cholangiocytes.

It is likely that each of the different types of PLC arises from some of the cells composing this lineal mixture of closely related hepatic epithelial cells, and that all PLC ultimately have a common cellular precursor. Current understanding of the mechanisms of cancer development from non-neoplastic precursor cells indicates that any cell that can undergo consecutive proliferative cycles is highly susceptible to neoplastic transformation from the effects of various carcinogenic agents (Weinberg 2006). Thus, in the pathologically damaged liver multiple, phenotypically varied epithelial cells, including mature, fully differentiated hepatocytes and cholangiocytes, and a diverse group of less completely differentiated intermediate cells and their precursors are susceptible to neoplastic transformation from exposure to various carcinogenic factors in their environments. In the remainder of this chapter we briefly review published literature that supports this opinion.

Although not widely influential, the idea that HCC and ICC are closely related tumors is not new. The existence of mixed PLC that contain morphologically distinct neoplastic hepatocytes and cholangiocytes was first described more than 85 years ago (Wells 1903) and comprehensively reviewed more than 60 years ago (Allen and Lisa 1949). The latter study conclusively demonstrated that many of these morphologically mixed tumors develop in the absence of separate ICC and HCC that might have collided during their growth, suggesting a common cellular origin. More than 50 years ago a seminal analysis of 100 PLC that included 65 hepatocellular and 21 cholangiocellular carcinomas (Edmondson and Steiner 1954), concluded that both neoplastic hepatocytes and cholangiocytes could often be morphologically identified in both types of PLC, noted the difficulty to discern unmistakable morphological evidence of either hepatocytic or cholangiocytic origin of the more undifferentiated carcinomas, hypothesized the derivation of HCC and ICC from a common cellular precursor, and suggested that these tumors be grouped together as hepatobiliary cancers, rather than being more specifically defined (Edmondson and Steiner 1954).

The importance of phenotypic properties other than morphology in distinguishing HCC and ICC was clearly demonstrated nearly 25 years ago in a study that applied the histochemical detection of selected cytokeratin proteins to the analysis of morphologically mixed HCC/ICC (Goodman et al. 1985), since cytokeratins expressed by mature hepatocytes and cholangiocytes differ in molecular type (Moll et al. 1982). Individual cells of mixed HCC/ICC (termed transitional carcinomas by the investigators) expressed a mixture of cytokeratins that blended those of fully differentiated hepatocytes and cholangiocytes, suggesting a unitary cellular origin for HCC and ICC (Goodman et al. 1985). This viewpoint was explicitly restated more than 10 years ago on the basis of similar studies that histochemically assessed these and other proteins that are differentially expressed in fully differentiated hepatocytes and cholangiocytes (D'Errico et al. 1996). Many other studies have since used histochemistry to examine selected molecular phenotypic properties characteristic of both mature hepatocytes and cholangiocytes in mixed HCC/ICC to confirm and extend these findings (Tickoo et al. 2002; Kim et al. 2004; others not cited due to space limitations). Some of these studies demonstrated that the prognosis of mixed HCC/ICC more nearly reflects that of ICC occurring separately (Jarnigan et al. 2001; Yano et al. 2003; Koh et al. 2005; Aishima et al. 2006). More recently expanded studies show that poorly differentiated PLC often express a mixture of hepatocyte, cholangiocyte, and precursor cell phenotypes (Kim et al. 2004).

Histochemical studies also show that a significant fraction of morphologically typical HCC expresses histochemically detected proteins characteristic of cholangiocytes, and that these tumors have a worse prognosis than HCC that does not express cholangiocyte phenotypes (Wu et al. 1996; Durnez et al. 2006; Aishima et al. 2007). Recent evidence demonstrates that the prognosis of morphologically characteristic HCC is determined by several complex phenotypes, or signatures (defined by global gene expression arrays), expressed by tumor cells (Lee et al. 2004a,b, 2006), including a gene signature detected in ICC (Woo et al. 2010). These studies show the importance of phenotypic analysis for accurate assessment of prognosis of PLC. The difficulty accurately to categorize the prognosis of PLC (both

well and poorly differentiated) by morphology alone poses a major problem for predicting prognosis and defining therapeutic strategy for these cancers, a dilemma that may be addressed by the analysis of the molecular phenotype of individual tumors.

Although it is commonly believed that HCC and ICC do not share major risk factors, recent epidemiological studies have identified common risk factors for both types of PLC (Shaib and El-Serag 2004; Bosch et al. 2005; Seeff and Hoofnagel. 2006; Shaib et al. 2005), additional evidence that PLC are closely related tumors. Most prominently, epidemiological studies suggest that chronic infections by hepatitis viruses B (HBV) and C (HCV), long known to be major risk factors for HCC, are also important risk factors for ICC (Yamamoto et al. 2004; Hai et al. 2005; Shaib et al. 2005; Welzel et al. 2006). Concordant with these epidemiological studies, assessment of the prevalence of markers for HBV and HCV infections in clinical studies of more than 4500 patients with PLC (Maeda et al. 1995; Yano et al. 2003; Koh et al. 2005; Chantajitr et al. 2006; Lee W et al. 2006; Tang et al. 2006; Zuo et al. 2007), detected HBV surface antigen in 41±30% of 3233 patients with HCC and in 18±10% of 292 patients with ICC, as well as in 39±18% of 282 patients with mixed HCC/ICC. In the same studies the prevalence of HCV antibody was 44±32% in patients with HCC, 16±10% in patients with ICC, and 29±26% in patients with mixed HCC/ICC. The prevalence rates of chronic hepatitis virus infection in each of these PLC is much higher than is reported in the general populations of the countries from which the data were collected, showing that hepatitis viral infections are potent risk factors for both types of PLC. Furthermore, HBV and HCV gene sequences have been found in ICC (Perumal et al. 2006), as in HCC (Edamoto et al. 1996).

Other potent risk factors also increase risk for both HCC and ICC. For example, intrahepatic deposition of thorium dioxide (as thorotrast, formerly but no longer used as an angiographic contrast medium), is a potent hepatocarcinogen as a consequence of its radioactivity and its accumulation in the liver (Sharp 2002). The risk for development of both ICC and HCC is increased to about the same extent in patients who were exposed to thorotrast during angiographic procedures (Sharp 2002). Likewise, genetic hemochromatosis (Morcos et al. 2001) and Wilson's disease (Ponomarev et al. 1994; Walshe et al. 2003), congenital metabolic diseases characterized by accumulation in the liver of excessive amounts of iron and copper, respectively, are associated with increased risk to both types of PLC. Patients with nonalcoholic steatohepatitis also appear to be at increased risk for both ICC and HCC (Ichikawa et al. 2006; Hashizume et al. 2007). Confirming the general biological application of these observations, both types of PLC are also produced by exposure of experimental animals to various hepatocarcinogenic chemicals that act by different molecular mechanisms (National Toxicology Program (1980–2009), various years), and by engineered genetic changes that alter diverse but specific molecular pathways (Lin et al. 1995; Horie et al. 2004; Xu et al. 2006; Kim et al 2007; Jang et al. 2007). Although certain risk factors are often associated with a dominant association with either HCC or ICC, the accumulated evidence suggests that virtually every known risk factor for PLC increases the risk of both HCC and IHC in both humans and in laboratory animals. The often larger number of

HCC relative to ICC may simply reflect the fact that hepatocytes far outnumber cholangiocytes in the total population of liver epithelial cells (Grisham 2009).

Substantial evidence also supports the opinion that ICC, HCC, and mixed HCC/ICC are closely related genomically. In studies employing identical methods to analyze loss of heterozygosity (LOH) at polymorphic microsatellite loci spanning the entire genome of typical HCC and typical ICC, unique LOH were found for each type of PLC, as well as LOH at specific loci that were shared by both ICC and HCC, suggesting significant overlap of genetic aberrations in these PLC (Momoi et al. 2001; Cazels-Hatem et al. 2004; Liu et al. 2004). Overlap of genomic aberrations in HCC and ICC is illustrated by comparison of the genomic locus losses and gains detected by CGH in 76 independent ICC included in four separate studies (Koo et al. 2001; Wong et al. 2002; Lee et al. 2004; Uhm et al. 2005) and in a meta-analysis of 785 HCC collected from 31 separate studies (Moinzadeh et al. 2005). Losses were found in more than 20% of HCC at loci on chromosome 4q, 8p, 13q, 16q, and 17p and gains were detected in more than 20% of HCC at loci on chromosome 1q, 6p, 8q, and 17q (Table 2.1). Locus losses were found in greater than 20% of ICC on 1p, 3p, 4q, 9p, and 17p, while locus gains were found in more than 20% of ICC on chromosome 1q, 3q, 5p, 6p, 7p, 8q, 12q, 15q, 17p, 17q, 18p,

Table 2.1 Comparison of chromosome losses and gains in HCC and ICC determined by comparative genomic hybridization (CGH)

Chromosome arm (Loss [–]/Gain [+])	HCC[a] (*n*=785) (%)	IHC[b] (*n*=76) (%)
1p–		30
3p–		20
4q–	34	26
8p–	38	
9p–		44
13q–	26	
16q–	36	
17p–	32	25
1q+	57	37
3q+		26
5p+		24
6p+	22	26
7p+		20
8q+	47	36
12q+		25
15q+		25
17p+		26
17q+	22	36
18p+		21
20q+		30

[a]From Moinzadeh et al. (2005); [b]From Koo et al. (2001), Wong et al. (2002), Lee et al. (2004), Uhm et al. (2005)

and 20q (Table 2.1). Twenty locus losses and gains occurred in both HCC and ICC combined. HCC and ICC shared locus losses on chromosome 4q and 17p and locus gains on chromosome 1q, 6p, 8q, and 17q. For total aberrations (losses and gains combined), 6 of 9 found in HCC were shared with ICC (67%), while only 6 of 17 losses and gains found in ICC (35%) were shared with HCC. Three of eight locus losses (located at 8p, 13q, and 16q) were unique to HCC (38%), but there were no unique locus gains in HCC (thus three of nine aberrations [both locus losses and gains] in HCC were unique [33%]). ICC had unique locus losses on chromosome arms 1p, 3p, and 9p (38%) and unique locus gains on chromosome arms 3q, 5p, 7p, 12q, 15q, 17p, 18p, and 20q (67%), for a total of 11 unique locus aberrations out of a total of 17 (65%). Of 20 loci that showed aberrations (both locus losses and gains) in the combined typical ICC and HCC, 6 (30%) were shared by both tumors, 3 (15%) were unique to HCC, and 11 (55%) were unique to ICC.

These data show that ICC and HCC have distinct genomic similarities, as well as significant genomic differences. The overlap of genomic aberrations in ICC and HCC is compatible with the concept that all PLC arise by differentiation from a common precursor cell. Genomic evidence derived from dissected components of mixed HCC/ICC suggests (but does not prove) that the ICC and HCC elements are clonal progeny of a single precursor cell (Imai et al. 1996; Fujii et al. 2000; Murata et al. 2001). A common cellular origin of both types of PLC is also supported by observations that cell lines cloned from PLC of both humans (Murakami et al 1987; Yano et al. 1996; Parent et al. 2004) and laboratory animals (Tsao and Grisham 1987; Gil-Benso et al. 2001) have the capacity to produce both HCC and ICC when transplanted into suitable hosts. Furthermore, stem-like cells (Tsao et al. 1984) with bipotential differentiation capacity (Coleman et al. 1994, 1997; Couchie et al. 2002) have been clonally isolated from the livers of healthy adult rats. When neoplastically transformed and re-cloned in vitro and subsequently transplanted into isogenic hosts, single clones of these neoplastic cells produce both HCC and ICC, as well as other types of PLC that express aberrant phenotypes, such as squamous (Tsao and Grisham 1987). Taken together these results reflect both the multipotential differentiation capacity of adult liver stem/progenitor cells and their neoplastic counterparts (Sell and Dunsford 1989).

These results can be explained most simply by the origin of PLC from a phenotypically plastic liver progenitor (stem) cell that can be isolated directly from the liver (in the instance of rodent liver epithelial cells) and/or from tumor stem cells (rodent and human liver tumor-derived cell lines) and that have multipotential differentiation capacities. Such results strongly support the idea that hepatocellular and cholangiocellular carcinomas are closely related tumors formed of cells with differentiation flexibility to express both hepatocellular and cholangiocellular phenotypes, and, therefore, the concept of the continuity of the liver epithelial lineage centered on bi- (multi-) potential stem cells. Although not discussed here, fibrolamellar carcinoma, a variant of HCC found mostly in young adults (Tanaka et al. 2005), hepatoblastoma, a PLC that occurs predominantly in children (Zimmermann 2003), clear cell carcinoma of the liver, sometimes considered to be a variant of either HCC or ICC (Adamek et al. 1998; Tihan et al. 1998; Olivera et al. 2000), and

rare tumors composed of epithelial cells that resemble liver stem/progenitor cells (Robrechts et al. 1998; Theise et al. 2003; Durnez et al. 2006) or intermediate cells (Kim et al.2004) are PLC that show mixed hepatocyte/cholangiocyte phenotypes and also likely arise from the hepatic epithelial cell lineages originating from fetal hepatoblasts and adult liver stem cells.

4 Conclusions

The prognosis of both ICC and HCC is grim. New methods to diagnose PLC at an earlier stage of development and to establish precise prognosis are needed to enable development of more adequate therapeutic strategies. Concepts and diagnostic/clinical practices pertaining to PLC need to be modified. The combined results of different types of studies that range from clinical to experimental suggest a new way to define PLC that may lead to more accurate diagnosis and more precise prognosis. Plausible changes would be to abandon the idea that ICC and HCC are distinctly different cancers and to base prognosis of both PLC on the phenotypic properties expressed by individual tumors. Much evidence now indicates that the complex phenotypes (gene signatures) expressed by individual PLC determine prognosis more accurately than does morphology.

The studies reviewed support the idea that cells of the entire hepatic epithelial lineage, originating from hepatoblast or adult liver stem cell, and including various intermediate cells, as well as mature hepatocytes and cholangiocytes, are susceptible to neoplastic change. The development of PLC is a cellularly and metabolically complex process that may originate from progeny of liver epithelial stem cells at different stages of differentiation and maturity to yield tumors that express phenotypes typical of mature, differentiated hepatocytes and cholangiocytes, and mixed phenotypes that blend elements of the phenotypes of hepatocytes, cholangocytes, and precursor cells. Neoplastic transformation of cells in the hepatocyte/cholangiocyte lineage appears to involve various combinations of genes and molecular regulatory pathways that characterize each fully differentiated cell and their precursors. Both well-differentiated HCC and ICC and a variety of tumors that express properties that differ from either of the major PLC, tumors either less completely differentiated or aberrantly differentiated, can result. Most important, prognostic characterization of the malignant neoplasms depends on the particular combination of genes and molecules expressed by individual tumors, and definition of relevant tumor-specific gene signatures is required. Gene signatures that contribute to the most adverse prognoses are already known to include those of the precursor cells of hepatocytes and cholangiocytes and some elements of the cholangiocyte phenotypes.

Although histochemical analysis of a limited number of molecular phenotypes may identify some of the phenotypic properties that are important for establishing prognosis of PLC, widespread use of this technique is impaired by methodological limits to specificity and sensitivity. Poor cellular differentiation further reduces

the sensitivity with which proteins characteristic of differentiated cells can be histochemically detected. Moreover, histochemical techniques are labor intensive and require relatively large amounts of tissue; the small size of tumor specimens typically available for diagnosis practically limits the number of phenotypes that can reasonably be analyzed in each tumor specimen by histochemical methods, preventing delineation of complex phenotypic signatures. Global analysis of gene expression by gene expression profiling (GEP) has supplanted histochemical methods as the most powerful and efficient technology currently available with which to assess the molecular phenotype and, thereby, the cellular origin and developmental stage of PLC. Most of this work has thus far been applied to analysis of HCC. Future application of GEP and other high-throughput techniques of genomic and phenotypic analysis to different tumors of the PLC spectrum may enable the identification of expressed gene signatures that characterize the prognosis of individual tumors throughout the entire spectrum of PLC.

(Note: Space limits prevented a comprehensive citation of the voluminous literature on these subjects. The authors apologize to the many investigators whose relevant work is not cited here.)

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