Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity

Vincenzo Cardinale, Guido Carpino, Lola Reid, Eugenio Gaudio, Domenico Alvaro

Abstract
Recent histological and molecular characterization of cholangiocarcinoma (CCA) highlights the heterogeneity of this cancer that may emerge at different sites of the biliary tree and with different macroscopic or morphological features. Furthermore, different stem cell niches have been recently described in the liver and biliary tree, suggesting this as the basis of the heterogeneity of intrahepatic (IH)- and extrahepatic (EH)-CCAs, which are two largely different tumors from both biological and epidemiological points of view. The complexity of the organization of the liver stem cell compartments could underlie the CCA clinical-pathological heterogeneity and the criticisms in classifying primitive liver tumors. These recent advances highlight a possible new classification of CCAs based on cells of origin and this responds to the need of generating homogenous diagnostic, prognostic and, hopefully, therapeutic categories of IH- and EH-CCAs.

INTRODUCTION
Cholangiocarcinoma (CCA) is an extremely heterogeneous cancer from a topographical, morphological, bio-
logical and clinical point of view. The two main forms, intrahepatic (IH)- and extrahepatic (EH)-CCAs, showed, in the last decades, opposite epidemiological behavior, with incidence and mortality progressively increasing for the IH form but stable or slightly decreasing for the EH form. This suggests different risk factors and, indeed, evidence exists that IH- and EH-CCAs may emerge from a different pathological background. A large body of recent literature deals with the role of stem cells in carcinogenesis. In this regard, different stem cell niches have been recently identified in the liver and biliary tree with new implications of the origin of primitive liver cancers. The complexity of the organization of the liver stem cell compartments could underlie the CCA clinical-pathological heterogeneity and well justifies the difficulties in clinical-pathological classification of primitive liver tumors. Another point of great consideration is the relationship between chronic liver damage and the development of primitive liver tumors. Indeed, the chronically injured liver and biliary tree could be considered a classic model of stem cell derived carcinogenesis where the activation and proliferation of the stem cell compartment in response to tissue injury represent the first step of the carcinogenetic process. In light of these recent advances, histological and morphological classification of primitive liver cancer is currently under revision, taking into consideration the potential cells of origin. Histological characterization of CCAs based on cell of origin responds to the need of generating homogenous diagnostic, prognostic and therapeutic categories or classes for IH- and EH-CCAs. The cells of origin of CCAs also represent the cell targets of the hepatic and biliary diseases associated with the development of CCAs and this could explain the association of determined hepatic and biliary pathologies with each category of CCA, other than the differences in terms of epidemiological, behavioral and risk factors.

**IH AND EH STEM CELL NICHES**

CCA arises from the lining epithelium and peribiliary glands (PBGs) of the IH and EH biliary tree and shows variable degree of differentiation. It has been recently elucidated how stem cells are particularly prone to be involved in the carcinogenic process due to their particular biological features. Stem cell niches have been firstly identified within the adult liver in the canals of Hering, remnants of the ductal plate of fetal and neonatal livers. Human hepatic stem cells (hHpSCs) residing in the canals of Hering can differentiate into hepatocytes and cholangiocytes lining the small bile ducts, which in turn can give rise to tumors with a wide range of phenotypes, with varying hepatocellular and biliary differentiation characteristics. More recently, additional stem cell niches have been identified in PBGs. PBGs are mucin-producing glandular elements located in the wall of the EH and large IH bile duct. Within PBGs, stem/progenitor cell niche composed of multipotent stem/progenitor cells of endodermal origin (biliary tree stem/progenitor cells: BTSCs) has been described, which represent the only cells potentially underlying the mucin-producing cells lineage within the liver. PBGs are distributed along the biliary tree starting intrahepatically at the level of septal-segmental bile ducts and ending at the hepatopancreatic common duct near the duodenum. PBGs are particularly high in density at the level of the cystic duct, hilum and periampular region, sites where EH-CCAs typically emerge. The IH PBGs are indistinguishable from the EH ones. For decades, the heterogeneity of IH and EH bile ducts has been the object of extensive investigations and the embryological origin of the biliary tree furnishes the basis for this topic. From an embryological point of view, indeed, the medium-large IH and the EH bile ducts recognize a unique origin and represent the result of the proliferation of the primitive endoderm at porta hepatitis. The interlobular bile ducts, in contrast, derive from SOX (SRY (sex determining region Y)-box) 9 positive progenitors following the re-organization of the ductal plate. Septal ducts (medium size), segmental ducts, larger IH bile ducts and EH bile ducts share unique histological features characterized by a discrete duct with a well defined wall constituted of connective tissue supporting the lining epithelia. Another unique feature joining the large IH bile ducts and the EH bile ducts is the presence in the duct wall of the PBGs. Furthermore, the large segments of the biliary tree share with EH bile ducts similar characteristic expression of cell markers both in physiological and pathological conditions that largely differ with respect to those observed in the interlobular bile ducts. Specifically, pancreatic α-amylase, trypsin and pancreatic lipase are expressed in biliary cells lining the PBGs in immature ducts in fetal livers and in mature large bile ducts in postnatal livers. Differently, these phenotypical markers disappear quickly when hHpSCs derived lineage restrict towards mature hepatocyte or cholangiocyte fates and interestingly expression is not found in developing peripheral IH bile ducts. Recently, we demonstrated that PBGs are stem cell niches in human EH bile ducts and that the maturation lineage progresses from the stem cells located in the deeper part of the wall, within the PBGs, to mature cells lining the surface epithelium. We also observed an increased mucin production with the maturation in the duct wall. The individuation of a stem cell niche within the bile ducts opens the possibility that BTSCs within PBGs and their descendant cells could be further considered as cells-of-origin of mucin-producing CCAs (Figure 1).

**CANCER STEM CELLS AND CANCER CELLS OF ORIGIN: DEFINITIONS**

The definition “cancer stem cell (CSC)” indicates the cellular subset within the tumor that uniquely sustains malignant growth. Differently, the term “cell-of-origin” defines the normal cell that acquires the first cancer-initiating
Cardinale V et al. Multiple cells of origin in cholangiocarcinoma

CANCER THERAPEUTIC TARGETS

LIVER CANCER STEM CELLS AND NEW CANCER THERAPEUTIC TARGETS

Many years of investigations and daily clinical practice suggest an alternative model of carcinogenesis where only a subset of CSCs has the ability to proliferate extensively and form new tumors. Signalling pathways associated with oncogenesis, including the Notch, Sonic hedgehog and Wnt signalling, play a major role in regulating stem cell self-renewal. The machinery for self-renewal is already activated in CSCs, thus fewer mutations may be required to maintain self-renewal than to activate it ectopically. Stem cells often persist for long periods of time, increasing the probability of mutations. CSCs tend to be more resistant to chemotherapeutics due to high levels of expression of multidrug resistance genes. As far as liver is concerned, intermediate carcinoma may be a distinct type of primary liver carcinoma, morphologically and phenotypically intermediate between hepatocellular carcinoma (HCC) and CCA, which originates from transformed hepatic progenitor cells. Moreover HCCs expressing biliary cell markers, such as keratin(K)7 and K19, have been demonstrated to carry a significantly poorer prognosis and have a higher recurrence rate after surgical resection and liver transplantation. A number of cell surface markers have been proved to be useful for the isolation of CSC enriched fractions in liver malignancies, including CD133 (also known as Prominin-1), CD44, CD24, epithelial cell adhesion molecule (EpCAM), α-fetoprotein, Thy-1 and ATP-binding cassette B5, as well as Hoechst13382 exclusion by the side population cells. Indeed, recently, it has been described that a poor prognosis characterizes HCCs expressing stem markers such as EpCAM and CD133. A correlation between the stage of hepatic differentiation and clinical manifestation, notably vascular invasion, metastatic spread and patient survival, was also established. Primary liver tumors might arise from impairment of the normal liver differentiation program associated with excessive
Wnt/β-catenin signaling\(^{[39]}\). Recently, EpCAM was identified as a direct transcriptional target of Wnt/β-catenin signaling in HCC\(^{[38]}\). A number of EpCAM-regulated target genes have been identified, including e-myc and cyclins, and additional genes involved in cell growth and proliferation, cell cycle and cell death\(^{[39]}\). These findings indicate that expression of EpCAM is strongly linked with proliferation of stem cells and that cancer development from CSCs may occur after aberrant EpCAM re-expression. Recently, it has been demonstrated that the induction of terminal differentiation of HCC CSCs by using oncostatin M is associated with a marked reduction of the proliferative properties of the cells and with enhanced sensibility to chemotherapy\(^{[40]}\). Finally, a subset of highly chemoresistant and invasive HCC CSCs with aberrant expressions of IL-6 and the transcription factor Twist has been recently described. This subset of CSCs displays regulated let-7 and miR-181 miRNA family members, where modulation of both miRNA dependent pathways can impact significantly on their biology\(^{[41]}\). Thus, the modulation of aberrantly expressed miRNA in HCC CSCs may be a useful strategy to limit CSC differentiation and invasion or improve responses to cytotoxic therapies. In different cancers, recent studies addressed potential strategies of treatment based on selective target of specific CSC\(^{[10]}\). Various therapeutic drugs that directly modulate CSCs have been examined in vivo and in vitro. However, CSCs clearly have a complex pathogenesis, with a considerable crosstalk and redundancy in signaling pathways, and hence targeting single molecules or pathways may have a limited benefit for treatment. Many of the key signaling molecules are shared by both CSCs and normal stem cells, which add further challenges for designing molecularly targeted strategies specific to CSCs. In addition to the direct control of CSCs, many other factors that are needed for the maintenance of CSCs, such as angiogenesis, vasculogenesis, invasion and migration, hypoxia, immune evasion, multiple drug resistance and radioresistance, should be taken into consideration when designing therapeutic strategies\(^{[10]}\). In CCA, these studies are only at the beginning and the heterogeneity of this cancer further hampers advancement. To this latter regard, obtaining clinical-pathological information capable of driving the diagnostic setting or the accurate realization of basic science studies pass through the mandatory acquisition of CCA tissue\(^{[15]}\).

### Table 1: Phenotype markers of candidate cholangiocarcinoma cells of origin

| Marker                        | Mature cholangiocytes | hHpSCs in canals of Hering | BTSC in peribiliary glands |
|-------------------------------|-----------------------|-----------------------------|---------------------------|
| Nanog, OCT4                   | -                     | -                           | +                         |
| CXC4                          | -                     | -                           | +                         |
| FoxA 2                        | -                     | -                           | +                         |
| PDX1, NGN3                    | -                     | -                           | +                         |
| AFP                           | -                     | -                           | +                         |
| Sox 9/17                      | -                     | +                           | +                         |
| Prominin-1 (CD133)            | -                     | -                           | +                         |
| EpCAM                         | *+/−                  | +                           | −/−                       |
| NCAM                          | +                     | +                           | +                         |
| Thy                            | +                     | +                           | +                         |
| CK7/19                        | +                     | +                           | +                         |
| Secretin Receptor             | +                     | +                           | −/−                       |
| γGT                           | +                     | *+/−                        | +                         |

Comparison of the phenotype among mature cholangiocytes, hepatic stem cells (hHpSCs) in canals of hering and biliary tree stem/progenitor cells (BTSC) in peribiliary glands. Mature cholangiocytes do not express markers of stem/progenitor cells. A complete study of the phenotype of cholangiocarcinoma considering these markers would indicate the probable cell of origin. AFP: α-fetoprotein; CD133: Prominin; CK: Cytokeratin; CXC4: CXC-chemokine receptor 4; EpCAM: Epithelial cell adhesion molecule; FoxA2: Forkhead box a2; γGT: γ-glutamitranspeptidase; NCAM: Neural cell adhesion molecule; NGN3: Neurogenin 3; OCT4: Octamer-binding transcription factor 4 also known as POU5F1 (POU domain, class 5, transcription factor 1); PDX1: Pancreatic and duodenal homeobox 1; SOX: Sry-related HMG box.

CCA CELLS OF ORIGIN AND NEW CCA CLASSIFICATION PROPOSALS

CCA is the primary malignancy of the biliary tract\(^{[38]}\). This cancer has been classified as either IH or EH, with the second-order bile ducts acting as the separation point\(^{[9]}\). Classically, EH-CCA has been divided into perihilar and distal CCA. According to the American Joint Cancer Committee/Union for International Cancer Control perihilar CCA is proximally separated from IH-CCA by the second-order bile ducts and distally separated from distal EH-CCA by the insertion of the cystic duct into the EH biliary tree\(^{[9]}\). CCA arises from the lining epithelium and peribiliary PBGs of the IH and EH biliary tree\(^{[18]}\). Recent studies further stressed the concept of CCA heterogeneity. The accurate comparison of lineage markers between normal and neoplastic cells can lead to individuate the cell-of-origin in different tumor subtypes arising within a given organ, even if tumor cells show phenotypical plasticity or dedifferentiate during neoplastic progression (Table 1). Therefore, lineage markers and molecular signatures of tumor cells may not precisely reflect the true cell-of-origin in normal tissue. The individuation of a stem cell niche within the bile ducts opens the possibility that BTSCs within PBGs could be further considered as cells-of-origin of mucin-producing CCA\(^{[14]}\) (Figure 1). Indeed, BTSCs represent the only cells potentially underlying the mucin-producing cells lineage within the liver. Strict similarities between the pathologies of biliary tract and pancreas have been recently suggested\(^{[18]}\); in response to injury, pancreatic duct glands undergo a mucinous metaplasia and have been indicated as a possible cell of origin of pancreatic cancer\(^{[18]}\). An unresolved question regarding CCA is which cell have to be considered as cells-of-origin (Figure 1). The involvement of different cells-of-origin could underlie CCA heterogeneity\(^{[27]}\). The most primitive cells, stem cells, are candidates for targets of transformation because of their self-renewal and longevity, which would allow the sequential accumulation of genetic or epigenetic mutations\(^{[27]}\). Two stem cell niches have been described within the liver: the Ca-
nals of Hering containing hHpSCs[13] and IH PBGs composed of BTSCs[14,15]. Nakanuma et al[18] recently proposed a new classification of CCA taking into consideration the heterogeneity of hepatic stem/progenitor cells and the pathological similarities between biliary and pancreatic neoplasms. The authors classified IH-CCA into: (1) bile ductular type or cholangiolocarcinoma (CLC); (2) intraductal neoplasm type; (3) conventional (bile duct) type and; (4) rare variants[19]. CLC is thought to originate from canals of Hering/bile ductules where hHpSCs are located. Komuta et al[17,40] showed that this subtype of CCA is mainly composed of CLC areas showing small monotonous and/or anastomosing glands, strongly positive for K7 and K19, with tumor boundary being characterized by a HCC-like trabecular area and with some cases expressing CCA areas with scarce mucin production. A comparison between CLC with K19-positive HCC and with combined HCC-CCA indicated a high homology[17,41]. The clear origin of CCL from hHpSCs or immediate descendent cells deserves high attention and the accurate histological observation of transitional zones within the tumor and the phenotype characterization are strongly recommended for a proper diagnosis. Following the maturation arrest theory, one could speculate that CCL represents the result of a carcinogenic process involving cells within the lineage derived from the hHpSCs and that the differentiation grade of the tumor reflects the grade of maturation of the cells primarily involved in the carcinogenesis process (Figure 1). In this view, the CCAs arising from the interlobular bile duct (small bile duct type CCAs) could be considered a tumor arising from differentiated cells belonging to the hHpSC-derived lineage (Figure 1). The evidence that IH-CCA and EH-CCA may be dissimilar tumors is supported by the recent discovery that, in vitro, they express diverse cellular proteins and have different cellular shape, doubling time, chromosome karyotype and chemosensitivity[40,41]. Similarly, researchers from France showed that hilar CCAs express higher levels of MUC5AC (60% vs 22%), Akt2 (64% vs 36%), K8 (98% vs 82%), annexin (56% vs 44%) and less vascular epithelial growth factor (VEGF) (22% vs 78%) as compared to IH-CCAs[42]. Moreover, prognostic markers were differentially expressed, as hilar CCAs carried out stronger perineural invasion (83% vs 42%) than peripheral CCAs[43]. The different biological and molecular features strongly support the concept that IH-CCA and EH-CCA arise from different carcinogenic processes and different cells-of-origin. Particularly relevant in the view of future clinical trials is the lower expression of VEGF in EH-CCA with respect to the IH-CCA, which could affect the response to anti-angiogenic based therapy. Relevant advantages in the way to a physio-pathological classification of the CCAs have been recently achieved by Komuta et al[17], who carried out a study aiming to investigate the CCA histological diversity in relationship to the heterogeneity of cholangiocytes lining the biliary tree: hilar mucin producing cells vs peripheral cuboidal ductular cells or hHpSCs. They investigated the clinical-pathological and molecular features of 79 resected CCAs and their relationship with hHpSCs and compared the spectrum of CCAs with respect to K19-positive or negative HCCs. According to this study, 52% of the IH-CCAs were pure mucin producing, whereas 48% showed mixed differentiation features, including focal hepatocytic differentiation and CCL features. CCAs with mixed features (mixed-CCAs) showed peripheral location, larger tumor size, less microvascular invasion, less lymph node involvement compared to pure mucin producing CCAs which showed perihilar location, smaller tumor size, more microvascular invasion and more lymph node involvement. S100p expression was seen only in pure mucin-producing CCAs, while NCAM expression was only present in mixed-CCAs and particularly in CCL. Molecular profiling showed high homology between mixed-CCAs and K19-positive HCCs (considered of hHpSCs origin). The authors concluded that mixed-CCAs and K19-positive HCCs have a similar molecular profile as the most peripheral ductules containing hHpSCs, while mucin producing CCAs have a similar profile to mucin producing large IH and EH bile ducts, possibly reflecting the different cells-of-origin[17]. Differences in clinical-pathological features between CCAs arising from small (interlobular bile ducts) or medium-large IH bile ducts are under investigations. Responding to the need for classifying IH-CCA in relationship to the heterogeneity of the small vs the medium-large IH bile ducts, recently Nakanuma et al[18] proposed to separately consider a small bile duct type (peripheral type) and a large bile duct type (perihilar type). The former is mainly described as a tubular or micropapillary adenocarcinoma while the latter involves the IH large bile ducts. In accordance with phenotypic differences between interlobular and medium-large bile ducts, Aishima et al[43] investigated 87 cases of IH-CCA smaller than 5 cm in diameter. They considered a hilar type IH-CCA, showing IH large bile duct involvement within the tumor, and a periphera type IH-CCA contained preserved architecture of the portal triad. They demonstrated that the frequency of perineural invasion, lymph node metastasis, vascular invasion, IH metastasis and EH recurrence of IH-CCA from large ducts was significantly higher than that of IH-CCA from small ducts[43]. The survival of patients with IH-CCA from large ducts was worse than that of patients with IH-CCA from small ducts[43]. In our hypothesis, the clinical-pathological differences observed among CCAs arising from small bile ducts and large bile ducts reflect the different lineage of origin, with the former arising from cells of the hHpSC-derived lineage and the latter arising from BTSC-derived lineage (Table 1, Figure 1). Also, the multiple lineages of origin could determine differences in signaling pathways or epigenetic mechanisms associated with the early phase of tumor development in the course of the hepatic and biliary diseases. By considering the process of maturation from the two different stem cell niches (canals of Hering and PBGs), one could expect that some IH-CCAs originate from cells within the lineage starting in the canals of...
These pathologies are characterized by a strong ductular reaction, a phenomenon involving interlobular bile ducts, bile ductules and canals of Hering, which is currently considered the morphological expression of the activation of the stem/progenitor cell compartment aimed to repair liver injury[55]. From the other side, pathologies of the large IH and EH bile ducts, such as liver flukes, cholangitis, PSC, choleodochal cysts, secondary biliary cirrhosis, choledocholithiasis, hepatolithiasis and cholecystitis, are characterized by the involvement of PBGs where cells proliferate and acquire the expression of stem cells and neuroendocrine markers (C-met, c-erbB-2, argirophil granules, chromogranin A)[52-54]. On the basis of these recent observations, we hypothesize that early cells within PBGs are the sites of origin of malignancies associated with chronic diseases or pathological conditions of the IH medium-large and EH bile ducts[8]. Differently, parenchymal liver diseases, including chronic viral and non-viral liver diseases and especially the liver cirrhosis, could be recognized as the associated diseases of the CCAs arising from the hHpSC derived lineage[8]. In complete agreement with this hypothesis, it has been recently demonstrated that viral hepatitis is associated with IH-CCA with cholangiocellular differentiation and N-cadherin expression[55]. We strongly believe that a lineage based classification of the CCAs could reveal the real strength of association of determined hepatic or biliary diseases with each class of CCA or resolve the bias of the spurious associations. Further studies are needed to corroborate these hypotheses which could explain the large different epidemiological profile of IH- and EH-CCA[8,9].

NEW INSIGHTS INTO LIVER STEM CELLS ORGANIZATION AND CCA DEVELOPMENT

Although the role of hHpSC in normal turnover of hepatocyte and cholangiocyte is still debated[44,45], the role of the stem cells during hepatic and biliary development has been recently better elucidated. Specifically, it was shown that interlobular bile ducts, during the early phase of embryological development, derive from the differentiation of hepatoblasts located closely to the forming portal tract (ductal plate) and is driven by the expression of SOX9[55]. Recently, Carpentier et al[46] came to the innovative result that the cells of the canals of Hering would be in direct communication and hierarchically derived from cells originating from the ductal plate. Ductal plate derived lineage should supply the epithelium of the inter- and intralobular bile ducts and passing through the canals of Hering should give rise to the perilobar hepatocytes. In this way, the development of the biliary tree and liver should proceed from the hilum to the periphery. Large IH bile ducts and EH bile ducts are very similar in terms of histology, cell phenotype and share common stem cells constituted by the BTSCs of the PBGs[44,45]. This is in keeping with their common embryological origin from a pancreatic-biliary progenitor [SOX17+/pancreatic and
The new scenario created by recent advances on liver stem cells suggests a physio-pathological classification of CCAs based on the cell-lineage-of-origin. Following the process of maturation from the two different stem cell niches, one could hypothesize that some IH-CCAs originate from cells within the hHpSC-derived lineage starting in the canals of Hering, while other IH-CCAs and the EH-CCAs could originate from the BTSC-derived lineage within the medium-large IH and EH bile ducts. The former could be classified on the basis of the grade of maturation of the cell-of-origin, in: (1) combined HCC-CCA (hHpSCs); (2) CCL or ductular type CCA (immature cholangiocytes); and (3) CCA of the small bile ducts or mixed-CCA (mature cholangiocytes of the interlobular bile ducts) (Figure 1). The latter could be classified in mucin-producing CCA of large bile ducts recognizing as cells-of-origin the ones within the BTSC-derived lineages (i.e., BTSCs in PBGs, cholangiocytes of large IH and EH bile ducts and goblet cells) (Figure 1). These observations are also supported by the similarities between the neoplastic pathologies of biliary tract and pancreas\(^{57}\). Indeed, most biliary and pancreatic neoplasias are of ductal lineage, characterized by tubule (gland), papillary structure formation and different degree of mucin production and expression of mucin-related glycoproteins\(^{57}\).

Classification of CCAs based on cells-of-origin needs definitive cells markers able to distinguish the CCA subtypes and thus it remains a challenge for future studies\(^{58}\). Meanwhile, however, it can be noted that the available diagnostic tools (imaging, clinical, histology) can suggest the presumptive cell-lineage-of-origin of the single CCA. Indeed, CCAs of the small bile ducts or mixed-CCAs, the hHpSC-lineage derived CCAs, usually show a peripheral localization and a mass forming growing pattern, while IH-CCAs of the large bile ducts, the BTSC-lineage derived CCAs, usually show a peri-ductal infiltrating and/or mass forming growth pattern and are hilar or peri-hilar and usually associated with IH bile duct dilatation and jaundice. From a histological point of view, CCAs of the small bile ducts or mixed-CCAs showed mixed differentiation features, including focal hepatocytic differentiation, ductular features and NCAM expression, while differently IH-CCAs of the large bile ducts are characterized by the involvement within the tumor of large bile ducts and of PBGs, mucin production and S100p expression. Taking into consideration this tentative of classification, risk factors and underlying pathologies associated with CCA development should be regarded in a new light. Parenchymal liver diseases, including chronic viral and non-viral liver diseases and liver cirrhosis, should be considered as risk factors for development of mixed-CCAs arising from the hHpSC-derived lineage, activated and expanded in the course of these pathologies. In contrast, chronic biliary diseases or pathologies and conditions affecting the IH medium-large and EH bile ducts are risk factors for CCAs of the large bile ducts since in these pathologies, stem cell niches located in the PBGs are activated. A physio-pathological classification of the CCAs according the cell-lineages-of-origin could have, in the

**CONCLUSION**

The new scenario created by recent advances on liver duodenal homeobox 1(PDX1)+, representing the result of the organization of the proliferation of the primitive endoderm at the porta hepatis. Recently, we demonstrated that remnants of these multipotent stem cells deriving from ventral endoderm are still present in the adult biliary tree, especially within PBGs\(^{13,14}\). The phenotype of the cells lining the large IH and the EH bile ducts or the PBGs in adult and fetal life\(^{13}\) and the recent insights supporting the bilum-to-periphery development of the biliary tree\(^{15,44}\), suggest that cells of the forming large IH bile ducts and EH bile ducts are precursors of the ductal plate cells in the fetal life. The new insights into liver stem cells niches organization further complicate the picture in relationship to the clinical-pathological classification of the primary liver cancers. Indeed, according to the innovative explanation of the liver organogenesis, the classical organization of the hepatic parenchyma stem cell niche, the canals of Hering, and the cell differentiation hierarchy within, are under debate. On the basis of these recent advances and analyzing the phenotype of the hHpSCs and of BTSCs (Table 1), an emerging hypothesis is that the BTSCs are precursors of the hHpSCs. This new scenario opens further perspectives in CCA classification. Indeed, based on this concept, the CCAs arising from the BTSC-derived lineage should be composed of tumor cells with earlier and endodermal-like phenotype with respect to hHpSC-derived CCAs. In this view, the anatomical localization of the CCAs, other than reflecting a different cell-of-origin, could be associated with different prognostic CCA classes. Indeed, it has been already demonstrated by different groups how the EH-CCAs (mostly hilar CCAs)\(^{17,43}\) and the IH-CCAs involving the large IH bile ducts are associated with the worst prognostic markers in comparison to the peripheral IH-CCAs involving the small bile ducts\(^{17,43,48}\). However, very recently, Andersen et al\(^{48}\) showed that discrete homogeneous molecular profiles of both hilar and peripheral-type CCAs were associated with different prognostic classes, suggesting that a similar molecular pathogenesis rather than the anatomical location defines the overall prognosis. However, IH-CCA could arise either from interlobular bile ducts/canals of Hering or from large IH bile ducts (segmental and septal), thus recognizing different cells-of-origin. Some of these cells-of-origin, the ones belonging to the BTSC-derived lineage, are the same for IH- and EH-CCA, while others, the ones derived from the hHpSCs, are specific for the IH-CCA (Figure 1). In our opinion, the different cells-of-origin potentially underlying the development of different IH-CCA subtypes should be taken into adequate consideration in defining prognostic classes of CCA (Figure 1). A gross classification based only on the anatomical localization and that considers all the IH-CCAs as a homogenous entity, does not reflect the complex biology of this cancer and could result in biases and criticisms.
future, important clinical implications with the definition of different prognostic classes or specific therapeutic targets. Given the differences in biology and clinical-pathology[19], IH-CCA with mixed features (mixed-CCAs or small bile ducts type) and mucin producing IH-CCA (large bile ducts type) should be considered separately (Figure 1).

REFERENCES

1 Callea F, Sergi C, Fabbretti G, Brisigotti M, Cozzutto C, Medicina D. Precancerous lesions of the biliary tree. J Surg Oncol Suppl 1993; 3: 131-133
2 Nakanuma Y, Minato H, Kida T, Terada T. Pathology of cholangiocellular carcinoma. In: Toke T, Kameda H, Okudaira M, Ohto M, Endo Y, Mito M, Okamoto E, Tanikawa K, Kojio M, editors. Primary liver cancer in Japan. Tokyo: Springer-Verlag, 1992: 39-50
3 Klatskin G. Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatitis, an unusual tumor with distinctive clinical and pathological features. Am J Med 1965; 38: 241-256
4 Nakanuma Y, Sripa B, Vatanasapt V, Leong ASY, Ponchon T, Ishak KG. Intrahepatic cholangiocarcinoma. In: Hamilton SR, Aaltonen LA, editors. World Health Organization classification of tumours: Pathology and genetics of tumours of the digestive system. Lyon: IARC Press, 2000: 173-180
5 Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene 2006; 25: 3818-3822
6 Welzel TM, McGlynn KA, Hsing AW, O’Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. J Natl Cancer Inst 2006; 98: 873-875
7 Alvaro D, Crocetti E, Ferretti S, Bragazzi MC, Caporaccia R. Descriptive epidemiology of cholangiocarcinoma in Italy. Dig Liver Dis 2010; 42: 490-495
8 Cardinale V, Semeraro R, Torrice A, Gatto M, Napoli C, Bragazzi MC, Gentile R, Alvaro D. Intra-hepatic and extrahepatic cholangiocarcinoma: New insight into epidemiology and risk factors. World J Gastrointest Oncol 2010; 2: 407-416
9 Reya T, Morrison SJ, Clare MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105-111
10 Oishi N, Wang XW. Novel therapeutic strategies for targeting liver cancer stem cells. Int J Biol Sci 2011; 7: 517-535
11 Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM. The canals of Herin and hepatic stem cells in humans. Hepatology 1999; 30: 1425-1433
12 Kuwahara R, Kofman AV, Landis CS, Swenson ES, Bardenswaard E, Theise ND. The hepatic stem cell niche: identification by label-retaining cell assay. Hepatology. 2008; 47: 1994-2002
13 Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, Melhem A, McClelland R, Turner W, Kulik M, Sherwood S, Tallheden T, Cheng N, Furth ME, Reid LM. Human hepatic stem cells from fetal and postnatal donors. J Exp Med 2007; 204: 1973-1987
14 Cardinale V, Wang Y, Carpinio G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology 2011; 54: 2159-2172
15 Carpinio G, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, Semeraro R, Anceschi M, Brunelli R, Alvaro D, Reid LM, Gaudio E. Biliary tree stem/progenitor cells in glands of extrahepatic and intrahepatic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J Anat 2012; 220: 186-199
16 Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G, Mendel G, Wauthier E, Barbier C, Alvaro D, Reid LM. Human hepatic stem cell and maturational liver lineage biology. Hepatology 2011; 53: 1035-1045
17 Komuta M, Govaere O, Vander Borth S, De Vos R, Vandecaveye V, Verslype C, Van Steenbergen W, Aerts R, Firenne J, Topal B, Nevens F, Desmet VJ, Roskams T. Histological diversity in cholangiocellular carcinoma suggesting different cells of origin: intrahepatic progenitor cells versus hilar mucin producing cells. J Hepatol 2011; 54 (Suppl 1): S37
18 Nakanuma Y, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. World J Hepatol 2010; 2: 419-427
19 Alison MR. Liver stem cells: implications for hepatocarcinogenesis. Stem Cell Rev 2005; 1: 253-260
20 Cardinale V, Wang Y, Carpinio G, Cui CB, Gatto M, Rossi M, Berloco PB, Cantafora A, Wauthier E, Furth ME, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid L. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology 2011; 54: 2159-2172
21 Alison MR, Islam S, Lim S. Stem cells in liver regeneration, fibrosis and cancer: the good, the bad and the ugly. J Pathol 2009; 217: 262-298
22 Nakanuma Y, Hosoi M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Microsc Res Tech 1997; 38: 552-570
23 Roskams T, Desmet V. Embryology of extra- and intrahepatic bile ducts, the ductal plate. Anat Rec (Hoboken) 2008; 291: 628-635
24 Tan CE, Moscoso GJ. The developing human biliary system at the porta hepatis level between 11 and 25 weeks of gestation: a way to understanding biliary atresia. Part 2. Pathol Int 1994; 44: 600-610
25 Antoniou I, Raynaud P, Cordi S, Zong Y, Tronche F, Stanger BZ, Jacquemin P, Pierreux CE, Clotman F, Lemaigre FP. Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology 2009; 136: 2325-2333
26 Terada T, Nakanuma Y. Expression of pancreatic enzymes (alpha-amylase, trypsinogen, and lipase) during human liver development and maturation. Gastroenterology 1995; 108: 1236-1245
27 Visvader JE. Cells of origin in cancer. Nature 2011; 469: 314-322
28 Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8: 755-768
29 Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 734-737
30 Bailleul B, Surani MA, White S, Barton SC, Brown K, Blessing M, Jocrano J, Balmain A. Skin hyperkeratosis and papilloma formation in transgenic mice expressing a ras oncogene from a suprabasal keratin promoter. Cell 1990; 62: 697-708
31 Brown K, Strathdee D, Bryson S, Lambie W, Balmain A. The malignant capacity of skin tumours induced by expression of a mutant H-ras transgene depends on the cell type targeted. Curr Biol 1998; 8: 516-524
32 Kim H, Park C, Han KH, Choi J, Kim YB, Kim JK, Park YN. Primary liver carcinoma of intermediate (hepatocyte-cholangiocyte) phenotype. J Hepatol 2004; 40: 298-304
33 Yamashita T, Ji J, Budhu A, Forgues M, Yang W, Wang HY, Jia H, Ye Q, Qin LX, Wauthier E, Reid LM, Minato H, Honda M, Kaneko S, Tang ZY, Wang XW. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/
progenitor cell features. Gastroenterology 2009; 136: 1012-1024

34 Yamashita T, Honda M, Nio K, Nakamoto Y, Yamashita T, Takamura H, Tani Y, Zen Y, Kaneko S. Oncostatin m renders epithelial cell adhesion molecule-positive liver cancer stem cells sensitive to 5-Fluorouracil by inducing hepatocytic differentiation. Cancer Res 2010; 70: 4687-4697

35 Meng F, Glaser SS, Francis H, DeMorrow S, Han Y, Passarini JD, Stokes A, Cleary JP, Liu X, Venter J, Kumar P, Priester S, Hubble L, Staloch D, Sharma J, Liu CG, Alpin G. Functional analysis of microRNAs in human hepatocellular cancer stem cells. J Cell Mol Med 2012; 16: 160-173

36 Yamashita T, Budhu A, Forgues M, Wang XW. Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. Cancer Res 2007; 67: 10831-10839

37 Schirmacher P, Bedossa P, Roskams T, Tiniakos DG, Brunt EM, Zucman-Rossi J, Manns MP, Galle PR. Fighting the bushfire in HCC trials. J Hepatol 2011; 55: 276-277

38 Blechacz B, Komuta M, Roskams T, Gores GJ. Clinical diagnosis and staging of cholangiocarcinoma. Nat Rev Gastroenterol Hepatol 2011; 8: 512-522

39 Farges O, Fuks D, Le Treut YP, Azoulay D, Laurent A, Bachelier P, Nuzzo G, Belghiti J, Pruvoit FR, Regimbeau JM. AJCC 7th edition of TNM staging accurately discriminates outcomes of patients with resectable intrahepatic cholangiocarcinoma: By the AFC-IHCC-2009 study group. Cancer 2011; 117: 2170-2177

40 Komuta M, Spee B, Vander Borch G, De Vos R, Verslype C, Aerts R, Yano H, Suzuki T, Matsuda M, Fujii H, Desmet VJ, Koijiro M, Roskams T. Clinicopathological study on cholangiocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 2008; 47: 1544-1556

41 He XR, Wu XP. Difference in biological characteristics and sensitivity to chemotherapy and radiotherapy between intrahepatic and extrahepatic cholangiocarcinoma cells in vitro. Chin Med Sci J 2008; 23: 54-59

42 Guedj N, Zhan Q, Perigny M, Rautou PE, Degos F, Belghiti J, Farges O, Bedossa P, Paradis V. Comparative protein expression profiles of hilar and peripheral hepatic cholangiocarcinomas. J Hepatol 2009; 51: 93-101

43 Aishima S, Kuroda Y, Nishihara Y, Iguchi T, Taguchi K, Taketomi A, Maehara Y, Tsuneyoshi M. Proposal of progression model for intrahepatic cholangiocarcinoma: clinicopathologic differences between hilar type and peripheral type. Am J Surg Pathol 2007; 31: 1059-1067

44 Carpentier R, Suher RE, van Hul N, Koopp JL, Beaudry JB, Cordi S, Antoniou A, Raynaud P, Lepreuix S, Jacquemim P, Leclercq IA, Sander M, LeMaigre FP. Embryonic ductal plate cells give rise to cholangiocytes, perportal hepatocytes, and adult liver progenitor cells. Gastroenterology 2011; 141: 1432-1438, 1438.e1-1438.e4

45 Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Koda M, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koiizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T, Uemoto S. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet 2011; 43: 34-41

46 Yoon SM, Gerasimidou D, Kuwahara R, Hytiorglou P, Yoo JE, Park YN, Theise ND. Epithelial cell adhesion molecule (EpCAM) marks hepatocytes newly derived from stem/progenitor cells in humans. Hepatology 2011; 53: 964-973

47 Heo J, Factor VM, Uren T, Takahama Y, Lee JS, Major M, Feinestone SM, Thorgerisson SS. Hepatic precursors derived from murine embryonic stem cells contribute to regeneration of injured liver. Hepatology 2008; 44: 1748-1486

48 Kimura W, Futakawa N, Zhao B. Neoplastic diseases of the papilla of Vater. J Hepatobiliary Pancreat Surg 2004; 11: 223-231

49 Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology 2011; 54: 173-184

50 Gaudio E, Carpino G, Cardinale V, Franchitto A, Onori P, Alvaro D. New insights into liver stem cells. Dig Liver Dis 2009; 41: 455-462

51 Terada T, Nakamura Y. Pathological observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers. III. Survey of necroinflammation and cystic dilatation. Hepatology 1990; 12: 1229-1233

52 Terada T, Nakamura Y. Pathological observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers: IV. Hyperplasia of intramural and extramural glands. Hum Pathol 1992; 23: 483-490

53 Terada T, Ashida K, Endo K, Horie S, Maeta H, Matsunaga Y, Takashima K, Ohta T, Kitamura Y. c-erbB-2 protein is expressed in hepatolithiasis and cholangiocarcinoma. Histopathology 1998; 33: 325-331

54 Terada T, Nakamura Y, Sirtica AE. Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. Hum Pathol 1998; 29: 175-180

55 Yu TH, Yuan RH, Chen YL, Yang WC, Hsu HC, Jeng YM. Viral hepatitis is associated with intrahepatic cholangiocarcinoma with cholangiolar differentiation and N-cadherin expression. Mod Pathol 2011; 24: 810-819

56 Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, Conner EA, Gillen MC, Roskams T, Roberts LR, Factor VM, Thorgerisson SS. Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. Gastroenterology 2012; 142: 1021-1031.e15

57 Nakamura Y. A novel approach to biliary tract pathology based on similarities to pancreatic counterparts: is the biliary tract an incomplete pancreas? Pathol Int 2010; 60: 419-429

58 Carpino G, Cardinale V, Reid L, Alvaro D, Gaudio E. Cells of origin and cancer stem cells in cholangiocarcinoma. Transl Gastrointest Cancer 2012; 1: 53-43

59 Faga V, Marzioni M, Benedetti A, Glaser S, DeMorrow S, Francis H, Alpin G. Molecular pathology of biliary tract cancers. Cancer Lett 2007; 250: 155-167

S- Editor Wang JL, I- Editor Roememele A, E- Editor Zheng XM