

# Immunohistochemical Analysis in Hepatocellular Carcinoma

## Does Age Matter?

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Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms in the world, and although it is considerably less common in the United States, an increase in the number of cases has been observed during the past 2 decades.<sup>1</sup> Studies from western Europe and Japan also have documented a rising trend in the incidence and mortality of HCC.<sup>2</sup> The incidence of HCC seems to be related to age; however, the age distribution varies in different geographic regions of the world. In the Western world, the increased incidence of HCC is observed only in patients older than 45 years, and the incidence continues to increase until 70 years of age, whereas in other parts of the world, HCC in people younger than 45 years is not uncommon.

Such differences may be explained by the difference in the age of exposure to hepatitis viruses. For example, in areas endemic for hepatitis B virus, exposure occurs at younger ages, and the incidence of pediatric HCC is higher. This association has been elucidated further by the experience of a nationwide hepatitis B vaccination program in Taiwan that resulted in a substantial reduction in the incidence of chronic hepatitis B infection among children younger than 15 years (93%) and HCC in children 6 to 14 years (50%).<sup>3</sup> Underlying liver disease is much less often a contributing factor in pediatric HCC in the United States. Although certain inborn errors of metabolism and congenital anomalies have been implicated in HCC in childhood, metabolic or other underlying liver diseases were lacking in most cases in a recent large study of the epidemiology of HCC in US pediatric population.<sup>4</sup>

So, do HCCs arising in older adults and children represent different entities? It is clear that most if not all HCCs observed in children in the United States represent de novo diseases that

do not arise in cirrhotic livers. In contrast, most adult HCCs arise in the background of cirrhosis. Previous study also has shown that the *c-met* mutation is identified only in children with HCC and the expression of cyclin D1 is much lower in pediatric HCC, whereas loss of heterozygosity on chromosome 13q is significantly more frequent.<sup>5</sup> These observations suggest there may be different pathways in hepatocarcinogenesis between adult and pediatric HCCs.

The cellular origin of HCC has been studied extensively in rodents. The model of diethylnitrosamine-induced hepatocarcinogenesis in the rat implies that HCC arises from mature hepatocytes. Long-term administration of furan products induces bile duct injury, ductular hyperplasia, intestinal metaplasia, and subsequent cholangiocarcinoma, as well as HCC, suggesting a role for bile duct progenitor cells. Notably, in this model, HCC is far less common than cholangiocarcinoma. In contrast, the Solt-Farber model—treatment with diethylnitrosamine followed by N-2-AAF and partial hepatectomy—selectively induced HCC, supporting the notion that bipolar ductular progenitor cells (oval cells) are the cells of origin for HCC.<sup>6</sup>

Although there are many similarities between the progenitor cell compartments of rodent and human livers, one must keep in mind that the rodent models and human pathology are not exactly comparable. Having said that, intermediate hepatobiliary cells, the rodent counterpart of oval cells in human liver, have drawn much attention in the fields of liver stem cell biology and hepatocarcinogenesis. These cells exhibit characteristics of both cholangiocytes and hepatocytes. Immunophenotypically, they express hepatocytic antigens (eg, HepPar1, albumin, and  $\alpha_1$ -antitrypsin) and biliary antigens (eg, cytokeratins [CK] 19, CK7, and OV6).<sup>7</sup> Expression of

CK7 and CK19 has been described in varying numbers of adult HCCs.<sup>8-10</sup> Despite this, the expression of CK7 and CK19 in pediatric HCCs has been studied only recently. The article in this issue of the *Journal* by Klein et al<sup>11</sup> is among the first to address this matter by histopathologic and immunohistochemical approaches.

The study by Klein et al<sup>11</sup> characterized the risk factors, histologic features, and immunohistochemical profiles of HCC in children and young adults. The authors also studied the histologic features of the background liver in HCC. All primary liver carcinomas from 1984 to 2003 in patients younger than 30 years were included in the analysis. A total of 23 cases were identified, including 13 HCCs, 9 fibrolamellar HCCs (FL-HCC), and 1 cholangiocarcinoma. Only 3 of 13 patients with typical HCC had known chronic hepatitis B infection, whereas none of the remaining patients had identifiable underlying liver diseases, congenital anomalies, or metabolic diseases. It is not surprising that all HCCs and FL-HCCs were positive for HepPar1. What is interesting is that 78% of HCCs and 100% of FL-HCCs were also positive for CK7, whereas only 37% of a group of control adult HCCs were similarly reactive.

There are several implications of this observation. First, these findings may be relevant to the discussion regarding the "cell of origin" in HCC. For example, does the CK7 staining in the HCC group studied by Klein et al<sup>11</sup> suggest an origin from intermediate hepatobiliary cells, ie, hepatic progenitor cells, or does it reflect a metaplastic phenomenon in malignant hepatocytes toward biliary differentiation? The result that HepPar-1 was uniformly positive in these HCCs favors the first explanation. Also, it is generally suggested (or hoped), that better differentiated neoplastic cells continue to express the CK profile of their cell of origin. The Solt-Farber model in animals showing that bipolar ductular progenitor cells give rise to HCC lends plausibility to this argument.

The second question then follows: What is the significance of the higher incidence of CK7 staining in the HCCs from this younger population? Does it suggest that HCCs arising at young age tend to derive from hepatic progenitor cells? This speculation seems to contradict the findings of previous studies suggesting that the extent of hepatic progenitor cell activation is correlated with the severity of the inflammatory infiltrate and the degree of fibrosis in various chronic liver diseases,<sup>12,13</sup> as most of the HCCs in the study by Klein et al<sup>11</sup> lacked an apparent etiology, both clinically and pathologically. The control group the authors used was tissue arrays containing 65 HCCs from adults older than 40 years. This control group is by no means perfect because heterogeneity is a common phenomenon in HCCs, and sampling issues in tissue arrays, therefore, are always a problem. Nevertheless, the prevalence (37%) of positive CK7 staining in the control adult HCC arrays was very similar to what had been reported previously (35%).<sup>14</sup>

The background liver diseases in these adult HCC arrays were not specified, but because the majority of the HCCs (including FL-HCCs) in the younger age group were not associated with identifiable underlying liver diseases, it would be interesting to know the exact prevalence and types of underlying etiology in the adult control population. It is possible that other factors besides chronic inflammation and fibrosis may transform hepatic progenitor cells. Klein and colleagues<sup>11</sup> suggest that the presence of germline mutations in one copy of a tumor suppressor gene might be a candidate for causality, referring to a small series of cases in children with adenomatous polyposis.<sup>15</sup> Although this is a tantalizing proposal, the cited report described only 2 cases, and no known inherited diseases or syndromes were found in the group of HCCs arising at young age in the group studied by Klein et al.<sup>11</sup> Hence, additional studies are needed to test this hypothesis further.

The finding of moderate to strong expression of CK7 in all 9 FL-HCCs also raises an intriguing question as to whether this particular variant of HCC shares similar pathogenesis with the classic HCC arising in younger individuals. FL-HCC tends to occur in the younger population and in livers without underlying diseases. In addition, immunohistochemical staining for CK19 is not as common in classic HCC or FL-HCC (22% and 0%, respectively, in the study by Klein et al<sup>11</sup>) when compared with that for CK7. In very early stages of liver regeneration, hepatic progenitor cells are immunoreactive for both CK7 and CK19. In later stages of regeneration, intermediate hepatocyte-like cells are positive for CK7 but lack CK19 staining, whereas CK19 is positive in the atypical reactive ductules and cholangiocytes.<sup>16</sup> These observations indicate that CK19 is a more restrictive marker for biliary differentiation. Does this immunohistochemical profile further define the cell of origin in HCC in younger patients? Obviously, more studies are required before we can answer this question.

The study by Klein et al<sup>11</sup> raises the question of whether CK7 reactivity in HCCs in children and young adults has any significant clinical implications. Do these lesions behave differently from those that are negative for CK7? Outcome studies exploring the possibility of different prognoses between CK7+ and CK7- HCCs in the young cohort are worthy of ongoing study. Finally, apart from the issues raised about hepatocarcinogenesis in younger individuals, does this article offer useful insights for day-to-day practice in diagnostic pathology? As concluded by the authors, HCCs in children and young adults frequently show positive CK7 immunoreactivity, something to keep in mind when CK7 is used in immunohistochemical panels designed to differentiate primary HCC from metastatic or pancreaticobiliary carcinomas.

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