EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

EpCAM, a new marker for cancer stem cells in hepatocellular carcinoma

Benoit Terris[1,2,3], Catherine Cavard[1,2], Christine Perret[1,2,*]

[1]*Institut Cochin, Département Endocrinologie Métabolisme et Cancer, Université Paris Descartes, CNRS (UMR 8104), Paris F-75014, France*
[2]*INSERM U567, Paris F-75014, France;* [3]*GHU Ouest, Groupe Hospitalier Cochin, Saint-Vincent de Paul, Hôpital Cochin, AP-HP, Service d'anatomie et de Cytologie Pathologiques, Paris F-75014, France*

EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. 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. Abstract reprinted from Gastroenterology 2009;136 (3):1012–1024 with permission from Elsevier.

Abstract: Background & Aims: Cancer progression/metastases and embryonic development share many properties including cellular plasticity, dynamic cell motility, and integral interaction with the microenvironment. We hypothesized that the heterogeneous nature of hepatocellular carcinoma (HCC), in part, may be owing to the presence of hepatic cancer cells with stem/progenitor features. Methods: Gene expression profiling and immunohistochemistry analyses were used to analyze 235 tumor specimens derived from 2 recently identified HCC subtypes (EpCAM(+) alpha-fetoprotein [AFP(+)] HCC and EpCAM(-) AFP(-) HCC). These subtypes differed in their expression of AFP, a molecule produced in the developing embryo, and EpCAM, a cell surface hepatic stem cell marker. Fluorescence-activated cell sorting was used to isolate EpCAM(+) HCC cells, which were tested for hepatic stem/progenitor cell properties. Results: Gene expression and pathway analyses revealed that the EpCAM(+) AFP(+) HCC subtype had features of hepatic stem/progenitor cells. Indeed, the fluorescence-activated cell sorting-isolated EpCAM(+) HCC cells displayed hepatic cancer stem cell-like traits including the abilities to self-renew and differentiate. Moreover, these cells were capable of initiating highly invasive HCC in nonobese diabetic, severe combined immunodeficient mice. Activation of Wnt/beta-catenin signaling enriched the EpCAM(+) cell population, whereas RNA interference-based blockage of EpCAM, a Wnt/beta-catenin signaling target, attenuated the activities of these cells. Conclusions: Taken together, our results suggest that HCC growth and invasiveness is dictated by a subset of EpCAM(+) cells, opening a new avenue for HCC cancer cell eradication by targeting Wnt/beta-catenin signaling components such as EpCAM.

The concept of cancer stem cells (CSCs) or tumour initiating cells (TICs) has been well established in several hematologic cancers, and has been extended more recently to solid tumours. This model predicts that a small subpopulation of cells, CSCs, maintains cancer tissues by sustaining phenotypically diverse cancer cells. These cells are able to self-renew and generate progeny cells, leading to cellular heterogeneity of the tumour. CSCs have a high level of resistance to chemotherapy and radiotherapy. A few of these cells may survive and lead to relapse of the disease (see [1] for review). This model may thus explain the extensive heterogeneity, high rates of recurrence in mortality, and the high level of resistance to conventional anticancer therapy observed in hepatocellular carcinoma (HCC) [2].

Yamashita et al. recently published an article addressing this issue [3]. They found a population of HCC cells expressing EpCAM, an epithelial cell adhesion molecule previously identified as a marker for stem/progenitor cells of adult liver and oval cells [4,5]. These cells seemed to present all the characteristics of CSCs. In a previous study, these authors identified new prognostic subtypes of HCCs by gene profiling. HCCs with the poorest prognosis were $EpCAM^+$ AFP^+ (α-foetoprotein) and showed hepatic cancer cell-like traits, whereas $EpCAM^-$ AFP^- HCCs had a more favourable outcome and displayed features of mature hepatocytes [6]. In the present study, the authors analyzed the EpCAM signature in more than 150 tumour samples, and confirmed that $EpCAM^+$ AFP^+ HCCs had a significantly shorter survival, with a higher frequency of portal vein invasion, than $EpCAM^-$ AFP^- HCCs. They also observed that EpCAM expression was concentrated at the invasive border of the tumour and thus may be a marker of cells with CSC properties. FACS analysis of hepatoma cell lines was used to separate two distinct populations of hepatoma cells on the basis of EpCAM expression levels. The authors showed that cells with high level of EpCAM ($EpCAM^+$) but not $EpCAM^-$ cells (with low level of EpCAM) expressed markers of hepatic stem cells, formed colonies in soft agar and efficiently formed non-adherent spheroid structures and were more invasive than the $EpCAM^-$ cells. When xenografted in NOD-SCID mice, only $EpCAM^+$ cells could efficiently initiate the development of invasive tumours, even after serial transplantation, demonstrating that $EpCAM^+$ cells display CSC-like characteristics. $EpCAM^-$ cells did not show these properties. These data are strongly supported by the analysis of NOD-SCID mice after transplantation of $EpCAM^+$ and $EpCAM^-$ HCC cells isolated from two fresh surgical specimens from patients with high serum AFP levels. Only $EpCAM^+$ cells were able to induce tumours in NOD-SCID mice.

* Corresponding author.
E-mail address: christine.perret@inserm.fr (C. Perret).

The gene encoding EpCAM is a target of the Wnt/β-catenin pathway [7], and this signalling pathway controls the proliferation of hepatic stem cells [8]. The authors thus showed that activation of β-catenin signalling by pharmacological approaches could increase the amount of spheroids formed by HuH7 cells. By contrast, inhibition by siRNA of either β-catenin or EpCAM led to a strong reduction in the capacity of cells to initiate tumours. These results show that EpCAM may serve as a marker for HCC CSC cells, and may be a potential new therapeutic target for HCC.

The work of Yamashita et al. highlights the link between EpCAM and the Wnt/β-catenin signalling pathway, but, as yet, whether β-catenin-activated HCC follows the CSC model remains unclear. Two different possible modes of activation of β-catenin signalling in human HCCs may be considered. The first mode of activation is the better characterized one, it results from activating mutations of the β-catenin gene that confer specific characteristics to HCCs, both in terms of their morphology and their expression pattern profiles [9,10]. In particular, β-catenin-mutated HCCs are usually largely homogeneous tumours with a well-differentiated pattern [9] and thus do not appear to fit with the CSC model. The second mode of β-catenin activation is not linked to any mutational events within the Wnt/β-catenin pathway, but results from cross-talk with other carcinogenic pathways (HGF, TGFβ and probably others), which stabilize β-catenin, and is likely to be involved in epithelial-to-mesenchymal transition during tumour invasion and metastasis [11,12]. Given the different levels of β-catenin activation in each of these two scenarios, the differences in the Wnt profile in these two types of HCC is not surprising. The $EpCAM^+$ signature described by Yamashita et al. is probably mainly associated with the second of these scenarios. This would explain the poorer prognosis observed for $EpCAM^+$ HCCs; β-catenin activation in this subtype of HCCs may promote both the hepatic stem/progenitor traits of these tumours and their invasiveness through EMT.

The identification of a subclass of $EpCAM^+$ HCC that displays CSC characteristics has important clinical consequences as these cells may be preferential therapeutic targets. It will also be important to determine whether the CSC model also applies to other subclass of HCCs. Indeed, a deeper understanding of the cellular organization in HCC is needed. However, this remains a difficult challenge due to the relatively poor efficiency of grafting HCC cells isolated from surgical specimens into immuno-deficient mice.

Acknowledgements

C. Perrt's group is supported by INSERM, CNRS, Université Paris Descartes, the Ligue Nationale Contre Le Cancer (Equipe labellisée 2008), ANR-07-Physio.

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