Intratumor Molecular and Phenotypic Diversity in Hepatocellular Carcinoma

Jean-Charles Nault (1,2,3) and Augusto Villanueva (4)

1. Inserm, UMR-1162, Génomique fonctionnelle des Tumeurs solides, IUH, Paris, F-75010, France
2. Université Paris Descartes, Labex Immuno-Oncology, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
3. Service d'Hépatologie, Hôpital Jean Verdier, AP-HP, Bondy, and Université Paris 13, Bobigny, France
4. Liver Cancer Research Program, Division of Liver Diseases, Division of Hematology and Medical Oncology, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, 10029

Corresponding Author: Augusto Villanueva, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave, Box 1123, Room 11-70, New York, NY 10029. Phone: 212-659-9392; Fax: 212-849-2574; E-mail: augusto.villanueva@mssm.edu

Running Title: Heterogeneity in Liver Cancer

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Hepatocellular carcinoma is a highly heterogeneous disease both at the molecular and clinical levels. Intra-tumor morphological and genetic heterogeneity adds a new level of complexity in our understanding of liver carcinogenesis, and it is likely an important determinant of primary and secondary resistance to targeted therapies.
In this issue of *Clinical Cancer Research*, Friemel et al. assessed the morphological and genetic heterogeneity of 23 hepatocellular carcinomas (HCC) (1). To this purpose, they performed geographical sampling of 120 different regions in this set of tumors. They collected the different pathological features and performed immunostaining to assess the activation of WNT/β-catenin pathway (β-catenin, glutamine synthase), the presence of stem cells markers (CK7, EPCAM and CD44), as well as CD34 and AFP expression. They also performed sequencing of *TP53* and *CTNNB1* (coding for β-catenin), two of the most frequently mutated genes in HCC. Morphological heterogeneity was observed in 87% of the cases and heterogeneity at the immunohistochemical (IHC) and morphological level in 61% of the cases. Strikingly, genetic intra-tumor heterogeneity was noted in 22% of the cases and was systematically associated with heterogeneity at both the pathological and IHC level. This suggested that, at least in these cases, genotype and phenotype were strongly correlated. Authors also showed how different *CTNNB1* mutations could be observed in different regions of the same tumor. As predicted, intra-tumor heterogeneity was more frequent in larger tumors suggesting that heterogeneity increases as tumor progresses. The authors postulate that genetic and phenotypic intra-tumor heterogeneity is frequent in HCC and questioned the use of single-biopsy to identify prognostic and predictive biomarkers.

Phenotypic heterogeneity has been widely described by pathologists. However, our understanding of genetic intra-tumor heterogeneity has been limited until recently due to our incomplete structural knowledge of the cancer genome, and to the lack of technology sensitive enough to easily detect tumor sub-clones. Luckily, the introduction of next-generation sequencing enabled scientist to draw the genetic landscape of the most frequent tumors. Moreover, it has allowed analytic refinements to identify low frequencies mutations and consequently to assess sub-clonality. As other cancers, HCC is a disease of the genome, where somatic mutations
affecting driver genes lead to a selective proliferative advantage. Mutations can also affect stochastically passenger genes without clear functional consequences in cell’s malignant phenotype. In addition, these genetic defects tend to accumulate in transformed hepatocytes. This phenomenon, also observed in almost all malignancies, is not linear what confers remarkable plasticity to tumor evolution (2, 3). In primary renal carcinoma, the pivotal study of Gerlinger et al. has shown that a minority of mutations was ubiquitous and shared between the different regions of the primitive tumor and the different metastatic sites (4). In contrast, some genetic alterations were exclusively shared by all the metastasis suggesting that a specific sub-clone within the main tumor harbored these metastasis-prone mutations. Strikingly, each part of the main tumor and of the different metastatic site harbored private mutations indicating that a continuous and independent mutational process persists. This study confirmed similar observations described concomitantly in other solid tumors and hematological malignancies (3).

A Darwinian theory has been proposed to describe the evolution of tumor clones that arose and diverge from the tumor cell considered as the most recent common ancestor. The selective pressure due to microenvironment, carcinogenic exposure and the acquisition of driver mutations led to the emergence of clones with growth advantage or metastatic ability (3). Selective pressure could also be exacerbated by treatment and emergence of clones harboring new mutations have been implicated in secondary resistance to targeted therapies (5). Consequently, tumor plasticity would become a major issue when designing second/third line therapies in advanced tumor stages. Besides genetic heterogeneity, other potential sources of phenotypic diversity within tumors are epigenetic changes (e.g., DNA methylation) and microenvironment (hypoxia gradients, local oxidative stress) (2). Considering that most HCC arise in the background of chronic liver disease with different levels of fibrosis and persistent inflammation, the impact of tumor microenvironment in intra-tumour heterogeneity surely
deserves further exploration (Fig. 1). Genetic intra-tumor heterogeneity has been barely evaluated in HCC and despite the relevant information provided by Friemel et al., the study leaves some unanswered questions. For example, they only focused on CTNNB1 and TP53 mutations but there are other somatic mutations identify using deep sequencing targeting ARID1A, ARID2, NFE2L2, KEAP1, RPS6KA3, MLL2, MLL3 or MLL4 for which there is no data regarding intra-tumor distribution (6). Moreover, we recently identify TERT promoter mutations as the most frequent somatic genetic defect in HCC, with an overall frequency of 60% (7). TERT promoter is also recurrently mutated in precancerous nodules. It is one of the earliest genetic alterations involved in malignant transformation in HCC and could be considered as a tumor “gatekeeper”. We could speculate that TERT promoter mutations are present in the common ancestor cell, being transmitted to its progeny and hence present in most tumor cells. As noted by the authors, further studies are still needed to decipher HCC intra-tumor genetic heterogeneity using unbiased approaches such as whole-exome or whole-genome sequencing, preferable by ultra-deep sequencing (2,3). Another issue raised by Friemel et al. is the reliability of molecular classification and genetic analysis using one single sample of resected tumors or using a single tumor biopsy. They suggest that tumor heterogeneity could challenge the use of molecular classification and development of biomarker driven targeted therapies in the field of HCC. However, such assumption needs to be balanced considering the available data. Several molecular prognostic signatures derived from the tumor have been validated in large and independent studies despite tumor heterogeneity (8,9). Also, all biomarker-based molecular therapies approved in solid malignancies are based on traditional genetic analysis of the tumor, that didn’t account for genetic heterogeneity. Some examples are vemurafenib for BRAF V600E mutated melanoma, cetuximab for wild type RAS colorectal cancer or crizotinib for ALK translocated non-small cell lung cancer.
The fact that independent tumor clones (“multicentric carcinogenesis”) can arise on cirrhotic livers adds an additional layer of complexity (Fig. 1). New data are required to delineate inter-tumor molecular heterogeneity and accurately distinguish between intrahepatic metastasis and multicentric carcinogenesis (i.e., de novo tumor) on a cirrhotic background. Until we understand the nature, mechanisms and consequences of this molecular diversity, it will be difficult to evaluate its impact in clinical practice. Accurate assessment of intra- and inter-tumor heterogeneity will be also pivotal to understand primary or secondary resistance to targeted therapies. Coupled with the analysis of the tumor genome, circulating tumor DNA will be an interesting tool to monitor tumor heterogeneity longitudinally and easily in clinical practice (10).

Grant Support

J.-C. Nault was supported by a fellowship from the Institut National du Cancer (INCa), the INCa with the ICGC, and the PAIR-CHC project NoFLIC (also funded by the ARC).

References


Figure 1. Schematic representation of intra and inter-tumor heterogeneity in hepatocellular carcinoma. Different areas of the main tumor show both molecular (i.e., mutations), and phenotypic (i.e., histology, immunohistochemistry) differences. Intra-tumor heterogeneity could complicate the identification of tumor drivers and mechanisms of resistance for second line therapies. Genetic and non-genetic factors have been implicated in intra-tumor heterogeneity. Additionally, cirrhotic livers can develop multi-clonal tumors, what increases the molecular complexity of multinodular hepatocellular carcinoma.
Figure 1:

Biopsy 1
- Mutation A
- IHC marker A

Biopsy 2
- Mutation A, B?
- IHC marker B

Biopsy 3
- Mutation A+B?, C?
- IHC marker C

Intratumor heterogeneity
- Identification of driver molecular events
- Mechanisms of resistance (second-line therapies)

Cirrhosis/fibrosis

Intertumor heterogeneity
- De novo HCC versus true metastasis
- Patterns of HCC recurrence (early and late)
- Impact on treatment response?

Genetic
- Clonal evolution

Nongenetic
- Epigenetic (e.g., DNA methylation)
- Microenvironment (e.g., metabolic stress, hypoxia)
Clinical Cancer Research

Intratumor Molecular and Phenotypic Diversity in Hepatocellular Carcinoma

Jean-Charles Nault and Augusto Villanueva

Clin Cancer Res  Published OnlineFirst January 27, 2015.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-2602

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.