

Next Generation of Preclinical Liver Cancer Models

Achim Weber¹, Tracy O'Connor², and Mathias Heikenwalder^{2,3}

Because of its heterogeneity, lack of prognostic markers, tumor-escape mechanisms, and frequent relapse upon surgical intervention, treatment of hepatocellular carcinoma (HCC) remains challenging. In this issue of *Clinical Cancer Research*, Groß and

colleagues characterize a rodent model that might help identify novel drugs for combinatorial sorafenib-based therapies for HCC. *Clin Cancer Res*; 21(19); 4254–6. ©2015 AACR.

See related article by Groß et al., p. 4440

In this issue of *Clinical Cancer Research*, Groß and colleagues (1) performed multiparametric multimodal imaging (MRI) and PET, and applied array CGH analyses to determine genetic heterogeneity and identify differences between commonly used rat hepatocellular carcinoma (HCC) model systems. HCC is the second most common cause of cancer-related death in humans worldwide and constitutes a major health problem in developing and industrialized countries. It comprises a clinically and pathologically heterogeneous spectrum of tumors with variable molecular and histologic changes. In general, HCC prognosis is still poor, despite massive efforts by experimental and clinical HCC researchers in the past years, and less than 40% of patients are currently eligible for curative treatments (e.g., liver resection or transplantation). Thus, the identification and development of appropriate treatments for particular HCC subtypes is imperative. Characterization of existing and generation of novel preclinical HCC rodent models recapitulating human disease and treatment response should help in selecting novel drugs for clinical trials.

Challenges for progress in HCC treatment are manifold, including the lack of clinically relevant and applicable molecular classifications of HCC for treatment stratification and the availability of suitable preclinical animal models that recapitulate pathology and characteristics of human HCC subtypes, as well as treatment responsiveness. Several attempts to classify HCC based on genetic, transcriptional, methylation, or miRNA levels have been made: Whole-exome sequence analysis of 250 HCC identified the most prevalent mutations (*TERT* promoter, *CTNNB1*, *TP53*, *ARID1A*, and *AXIN1*) and signatures associated with HBV infection, alcohol intake, tobacco, and other genotoxic substances (2). HCC gene expression studies identified tumor subgroups G1–G6, each associated with particular clinical and genetic parameters, including chromosome stability, with G1–G3 tumors being unstable

and G4–G6 tumors being more stable (3). A recent meta-analysis of 603 HCC patients demonstrated that common transcriptome-based subclasses exist across multiple studies, supporting the idea that there is a commonality in the global molecular status of HCC, irrespective of the heterogeneity in worldwide populations (4). The subclasses of aggressive tumors (termed S1 and S2) associated with a larger tumor size and poor histologic differentiation corresponded to the "proliferation class" with poor survival. The "proliferation class" was characterized by activation of the Notch signaling pathway, while mutations in the *CTNNB1* gene were enriched in the "nonproliferation class" (S3). This provided the basis for studying the molecular background of each HCC class. However, these and other classification proposals have largely failed to be integrated into clinical practice for the management of HCC patients—the ultimate goal of a clinically useful classification.

In 2008, significant progress was achieved when increased survival of patients with advanced HCC was reported following treatment with sorafenib (Nexavar) in a phase III trial (5). Based on these studies, stratification of patients who benefit from sorafenib treatment (6) was initiated, and a mechanism of sorafenib resistance in liver cancer (7) was identified. It is expected that improved patient stratification, as well as combinatorial treatments, might enhance the efficacy of sorafenib. Another challenge for the development of HCC treatment is the substantial degree of tumor heterogeneity, present not only within the tissue but also on an intratumoral level (8).

Another limitation is the lack of suitable preclinical rodent models in which to test mono- or combinatorial treatments. Ideally, preclinical models should faithfully reflect the complexity and heterogeneity of human pathology. Several rodent HCC models recapitulate features of chronic liver disease caused by chronic inflammation, genotoxic (9) or metabolic stress (10, 11). However, it has become apparent that most rodent models reflect in most cases "only" particular features found in certain subtype (s) of human HCC—and that treatment success in these models does not necessarily correlate with successful translation to the clinics. Thus, existing as well as future rodent models have to be stratified more thoroughly on histologic, genetic, and molecular levels, as well as for their responsiveness to different treatments. Moreover, these parameters have to be correlated with human HCC in order to identify which human HCC subtype they most closely resemble, and for which treatments they could be used as a preclinical model.

¹Institute of Surgical Pathology, University Hospital Zurich, Zurich, Switzerland.

²Institute of Virology, Technische Universität München (TUM)/Helmholtz Zentrum München (HMGU), Munich, Germany.

³Division of Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany.

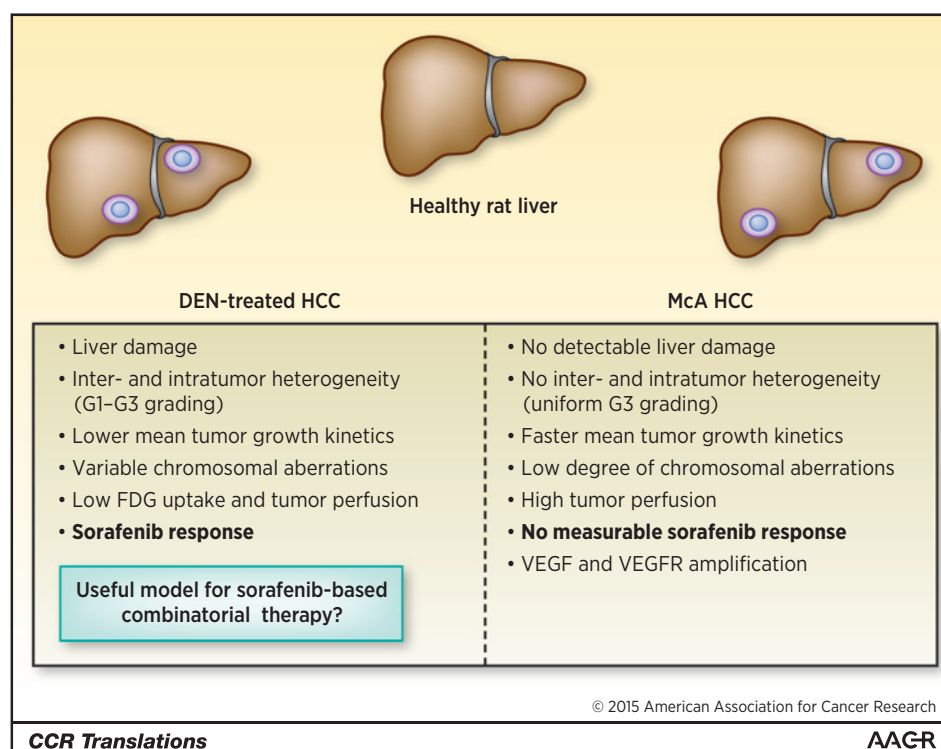
Corresponding Author: Mathias Heikenwalder, Schneckenburgerstrasse 8, 81675 München, Germany. Phone: 49-89-4140-7440; Fax: 49-89-4140-7444; E-mail: heikenwalder@helmholtz-muenchen.de

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Figure 1.

Differences of di-ethyl-nitrosamine treated and the McA HCC rat models and their possible usefulness for further studies on sorafenib-based combinatorial treatment. Groß et al. (1) show that DEN-treated rats are sorafenib responsive and fulfill the criteria to be used for future preclinical trial testing of several drugs in a sorafenib-based combinatorial therapy.



In the current study, Groß and colleagues induce HCC in rats using two different methods: one induced by di-ethyl-nitrosamine (DEN) and the other induced by orthotopically implanted (McA) rat HCC (Fig. 1). Based on histology, genetic analyses, and multimodal imaging, they found liver damage only in DEN-treated rats. Moreover, DEN-induced liver tumors displayed G1–3 grading compared with the uniform G3 grading found in McA tumors. Moreover, DEN tumors exhibited lower mean growth rates and FDG uptake and higher diffusion and perfusion values than McA tumors. Finally, DEN-induced tumors were responsive to sorafenib treatment, whereas orthotopically implanted (McA) rat HCC were not. These findings indicate important differences in treatment responses between model systems, and thus might be of translational relevance. Further, based on the outcome of sorafenib monotherapy, the data suggest that the rat DEN model might be suitable for future testing of novel combinatorial therapy including sorafenib. They further underline that thorough analysis and comparison to human pathology has to be performed in order to get a realistic assessment of the applicability of each rodent models. Moreover, the outcome of ongoing and completed clinical trials with human HCC patients using sorafenib combination therapy should be compared with the DEN rat model. Similar responses in the DEN rat model and human trials would further validate the human relevance of this model. Moreover, the mouse DEN model should also be investigated, given its amenability to gene manipulation and responsiveness to sorafenib (12).

Still, the study by Groß and colleagues leaves some open questions: (i) DEN is a chemical carcinogen and thus reflects only particular HCC etiologies (e.g., toxin-induced). DEN treatment hardly recapitulates a chronic liver disease state characterized by persistent liver damage, compensatory proliferation, and subsequent chromosomal aberrations, the background on which

the majority of HCC arise. (ii) Interestingly, although amplification of VEGF or VEGFR is reported to be a stratification criterion for a more efficient therapy in human patients (e.g., long-term survival), it is the orthotopically implanted (McA) rat HCC (sorafenib nonresponders) that predominantly display amplification of VEGF or VEGFR. (iii) Although partial treatment responses were reported for sorafenib in the DEN model, it has not been identified which (a) molecular changes (e.g., BRAF point mutations having been reported in a subpopulation of DEN-induced murine liver tumors), or (b) which histologic features were related to sorafenib responsiveness. (iv) As stated by the authors, different rat strains and sorafenib treatment regimens were used in this study for DEN and McA. Thus, the question remains whether longer sorafenib treatment or earlier treatment start might have also induced a response in the McA model.

In summary, this study underlines that stratification and comparison of rodent models with human HCC is important in order to validate their applicability, although this might be difficult in most cases and may never fit perfectly to a particular human subtype. Importantly, this study also shows that treatment response can be a critical stratification criterion, potentially providing a basis for future validation experiments (e.g., combinatorial treatment)—that ultimately might be more clinically relevant. Thus, the study by Groß and colleagues is an important first step in establishing and identifying useful preclinical rodent models for HCC research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Weber, M. Heikenwalder

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Heikenwalder

Writing, review, and/or revision of the manuscript: A. Weber, T. O'Connor, M. Heikenwalder

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References

- Groß C, Steiger K, Sayyed S, Heid I, Feuchtinger A, Walch A, et al. Model matters: differences in orthotopic rat hepatocellular carcinoma physiology determine therapy response to sorafenib. *Clin Cancer Res* 2015;21:4440–50.
- Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Calderaro J, Rebouissou S, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. *Nat Genet* 2015;47:505–11.
- Zucman-Rossi J. Molecular classification of hepatocellular carcinoma. *Dig Liver Dis* 2010;42:S235–41.
- Villanueva A, Llovet JM. Impact of intra-individual molecular heterogeneity in personalized treatment of hepatocellular carcinoma. *Hepatology* 2012;56:2416–9.
- Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–90.
- Horwitz E, Stein I, Andreozzi M, Nemeth J, Shoham A, Pappo O, et al. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. *Cancer Discov* 2014;4:730–43.
- Rudalska R, Dauch D, Longerich T, McJunkin K, Wuestefeld T, Kang TW, et al. In vivo RNAi screening identifies a mechanism of sorafenib resistance in liver cancer. *Nat Med* 2014;20:1138–46.
- Friemel J, Rechsteiner M, Frick L, Bohm F, Struckmann K, Egger M, et al. Intratumor heterogeneity in hepatocellular carcinoma. *Clin Cancer Res* 2015;21:1951–61.
- Ringelhan M, Reisinger F, Yuan D, Weber A, Heikenwalder M. Modeling human liver cancer heterogeneity: virally induced transgenic models and mouse genetic models of chronic liver inflammation. *Curr Protoc Pharmacol* 2014;67:14.31. 1–14.31.17.
- Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, et al. Metabolic activation of intrahepatic CD8⁺ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell* 2014;26:549–64.
- Nakagawa H, Umemura A, Taniguchi K, Font-Burgada J, Dhar D, Ogata H, et al. ER stress cooperates with hypernutrition to trigger TNF-dependent spontaneous HCC development. *Cancer Cell* 2014;26:331–43.
- Kapanadze T, Gamrekelashvili J, Ma C, Chan C, Zhao F, Hewitt S, et al. Regulation of accumulation and function of myeloid derived suppressor cells in different murine models of hepatocellular carcinoma. *J Hepatol* 2013;59:1007–13.

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