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Methodological Heterogeneity in Hepatocellular Carcinoma Research: Where Are We?

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COLUMN ARTICLE

Accurate re-creation of disease state to recapitulate clinical signs and the pathological features of the disease in question is indispensable not only for search for novel therapeutic agents against the disease but also guarantees the reproducibility of efficacy. This is to say that failure to accurately re-create the disease state experimentally may create recipe for irreproducibility of target endpoints. Among other factors, the high heterogeneity in models may account for the growing disconnect between pre-clinical and clinical efficacy of candidate drugs. Caught in this quagmire are models for hepatocellular carcinoma (HCC) research.

Cell models designed to study human HCC offer a fine platform for advancing knowledge and understanding needed to characterized therapeutic strategies against human HCC. Diverse humanized HCC cell lines normally used in *in vitro* studies include HepG2, HepG3, Hep3B, HepG4, and H-4, Huh7, HuH6, SK-HEP-1 and L02, HuH-7, Li-7, PLC/PRF/5, HLF, and HLE. Recently, precision cut liver slices (PCLS) have been introduced as alternatives to *in vitro* models of ALD and HCC [1]. Although theses cell models are useful with respect to investigating phenotypic hallmarks of HCC such as cell proliferation, migration, invasion, and apoptosis, nonetheless they are without drawbacks.

For example, they are not suitable for studying some aspects of HCC, particularly the molecular mechanisms and diagnostic/therapeutic interventions, due to the absence of the peculiar cellular microenvironment.

Animal models of HCC afford the opportunity to study efficacy and molecular mechanisms involved in HCC pathogenesis. These *in vivo* models are categorized based on the agent used to establish the disease. For example, human HCC can be induced in experimental animals using a variety of chemicals [2-9]. Among the considerations for choosing a particular chemical as inducing agent include: liver-specificity, specificity of inducing agent to liver cancer subtype, wide window of route of administration, duration of induction, reproducibility, dose-dependency, time dependency, ability of inducing agent to induce HCC in different experimental animals, and ability of inducing agent to afford study of many signaling pathways implicated in HCC. Generally, it is practically impossible to get an inducing agent that possesses all the properties to be able to accurately produce liver cancer subtype of choice. For example, diethyl nitrosamine (DEN) also known as N-nitrosodiethylamine, preferentially used to induce HCC [10-14] skips some crucial pathological events such as steatohepatitis, fibrosis, and cirrhosis reminiscent of human HCC. The time course of human HCC may span two decades or more, though this may be shortened by the number and severity of etiology exposure as well as genetic disposition. One major deficiency of chemically induced HCC animal models is the mechanism by which they induce HCC as well as the duration of induction. Implicit in this is that results from pre-clinical studies with respect to HCC may not be repro-

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ducible in clinical studies.

Other animal models include: infection-associated (HCV core transgenic mouse, HCV core, E1, E2 transgenic mouse, chronic *H. hepaticus* infected mouse, HBx/c-Myc transgenic mouse, HBV, LHBs transgenic mouse, HBV HBx transgenic mouse); inflammation-associated (Mdr2 knockout mouse, fatty liver shionogi [FLS] mouse, hepatocyte - specific conditional NEMO knockout mouse [$NEMO^{LPC-KO}$]); carcinogen-induced (Diethyl nitrosamine [DEN] treated rodents, choline deficient diet, carbon tetrachloride [CCl_4] treated rodents); transgenic mouse models (SV40-antigen transgenic mouse, β catenin/H-ras double transgenic mouse, TGF-α/c-Myc double transgenic mouse, c-Met conditional knockout mouse [MetLivKO]); and others (Immunotolerized rat model, chimeric mouse model, uPA/SCID mouse model, and GBV-B). Aside these models of HCC, there are etiology-specific models such as those of alcohol and LPS.

The argument has always been made that since more than two thirds of HCC incidence is causally associated with hepatitis B and C viral infections, HBV-and HCV-induced rodent models of HCC must be used as standard models of HCC. In as much as this may be partly understandable, many legitimate issues remain. For instance HCC is caused by multiple etiologies which in most cases work synergistically. How can unique individual contributions of each etiological factor be produced by one model? Is there a window of intense contribution to HCC pathogenesis that reflects kind of etiology? If yes, do current models of HCC incorporate this? What manifest as human HCC is a product of many etiologies acting in complex ways that cannot be easily replicated in an experimental model.

As we look into the future efforts must focus on designing new experimental models of HCC that will substantially address the deficiencies of existing *in vitro* and *in vivo* models and at the same time take into account the multi-etiology, time course and complex pathology of HCC. At least such an ideal model can accurately recreate pathological and molecular features of human HCC. This may in a way close up the usual disconnect between pre-clinical and clinical studies with respect to efficacy of newly discovered small molecules (NDSMs) with potential anti-HCC effects.

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