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Editorial

Molecular targeted therapies in hepatocellular carcinoma: From pre-clinical models to clinical trials ☆

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the world's most common and deadly cancers. Less than one-third of patients can currently benefit from potentially curative therapies in the West [1]. However, a new era has dawned in oncology with novel and promising drugs emerging in parallel with a better understanding of the pathogenesis of cancer [2].

The advent of sorafenib – a multikinase inhibitor – as an effective therapy in advanced HCC has enhanced the interest in testing new molecular therapies in experimental and clinical studies [3]. Integrative genomic studies in human HCC samples have begun to identify subgroups of patients with characteristic molecular features such as mutations, gene expression profiles and chromosomal aberrations [4,5]. These studies have underlined the fact that a number of molecular pathways are disrupted in almost all tumors, involving critical functions for the progression or dissemination of the disease. Such is the case of three main cellular functions: (1) Activation of pro-angiogenic signals mediated by VEGF, PDGER, angiopoeitin-2, and others [4,6]; (2) Mitosis checkpoint disruption and activation of pro-apoptotic mediated by mutations of critical tumor suppressors (e.g. p53, inactivation of Rb) or activation of oncogenes (e.g. Cyclin D1) [7]; (3) Acquisition of limitless replicative potential through the activation of TERT at pre-neoplastic and early HCC stages [8]. Nonetheless, the main driving force ensuring tumor viability in HCC depends on the activation of specific signal transduction pathways leading to tumor proliferation. Nonetheless, the main driving force ensuring tumor viability in HCC depends on the activation of specific signaling pathways leading to tumor proliferation. From the molecular classifications published so far, one-third of HCCs are driven by proliferative signals generated from Tyrosine Kinase Receptor (e.g. EGFR, IGF-IR), RAS/MAPK, PI3K-Akt-mTOR or c-MET signaling transduction pathways [5,9,10]. In another third of HCC patients, cell proliferation is lead by activation of Wnt pathway, mostly as a result of β-catenin mutations [11,12]. Genomic abnormalities driving proliferation in the remaining cases are still unclear. Therefore, there is rationale to combine drugs abrogating potent signals at different levels of one of the main pathways (e.g. blocking EGFR with erlotinib and Raf-Ras with sorafenib) or abrogating signals of two different pathways (e.g. VEGF with bevacizumab and mTOR with rapamycin, as in the study by Huynh et al.) [13]. Since there is not a single dominant molecular pathogenesis underlying all HCCs, it is increasingly clear that different models will be ultimately required to mimic different subclasses of the neoplasm.

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Investigators at the front-line of drug development of targeted therapies in HCC are now facing two challenging questions. First, what is the best experimental model to assess new molecular targeted therapies in HCC, and second, if there are data to support a direct correlation between experimental findings and clinical outcomes in phase II–III studies in oncology and HCC.

2. Testing new drugs in pre-clinical HCC models

The demonstration that concentrated cancer cells grown *in vitro* could form tumors when implanted subcutaneously into an immunocompromised mouse was first established in 1969 [14]. This xenograft model has since demonstrated several advantages that explain its persistence as the mainstay of pre-clinical studies of antineoplastic drugs *in vivo*: the tumors are rapidly and easily induced, and their subcutaneous location enables direct measurement of tumor growth. More recently, however, several critical differences between xenograft- and patient-derived specimens have become apparent. Cancer is now appreciated as a complex disease dependent upon the interaction between transformed cells harboring oncogenic mutations and their surrounding tumor environment made up of normal cells, stromal cells, and immune cells [15].

One of the challenges we face in pre-clinical testing of targeted therapies in HCC is the lack of models that accurately recapitulate the disease in humans. Several key mouse models have been instrumental in defining the pathogenesis of HCC by introducing genetic alterations into one or more etiologic pathways that can be targeted exclusively to the liver [16]. Nonetheless, substantial challenges persist in modeling liver diseases whose natural history requires a chronic inflammatory milieu. Although these genetically modified mice have been employed to investigate the molecular pathways dysregulated in HCC, they are not commonly employed for pre-clinical drug testing, using either cytotoxic chemotherapeutic or molecularly targeted agents [16].

Pre-clinical testing, in HCC as in the majority of cancers, is typically performed in immune deficient mice using human tumor xenografts grown subcutaneously [16,17]. In the study published this month in the Journal by Huynh et al., the authors assess the efficacy of Bevacizumab and Rapamycin in two different nude mouse

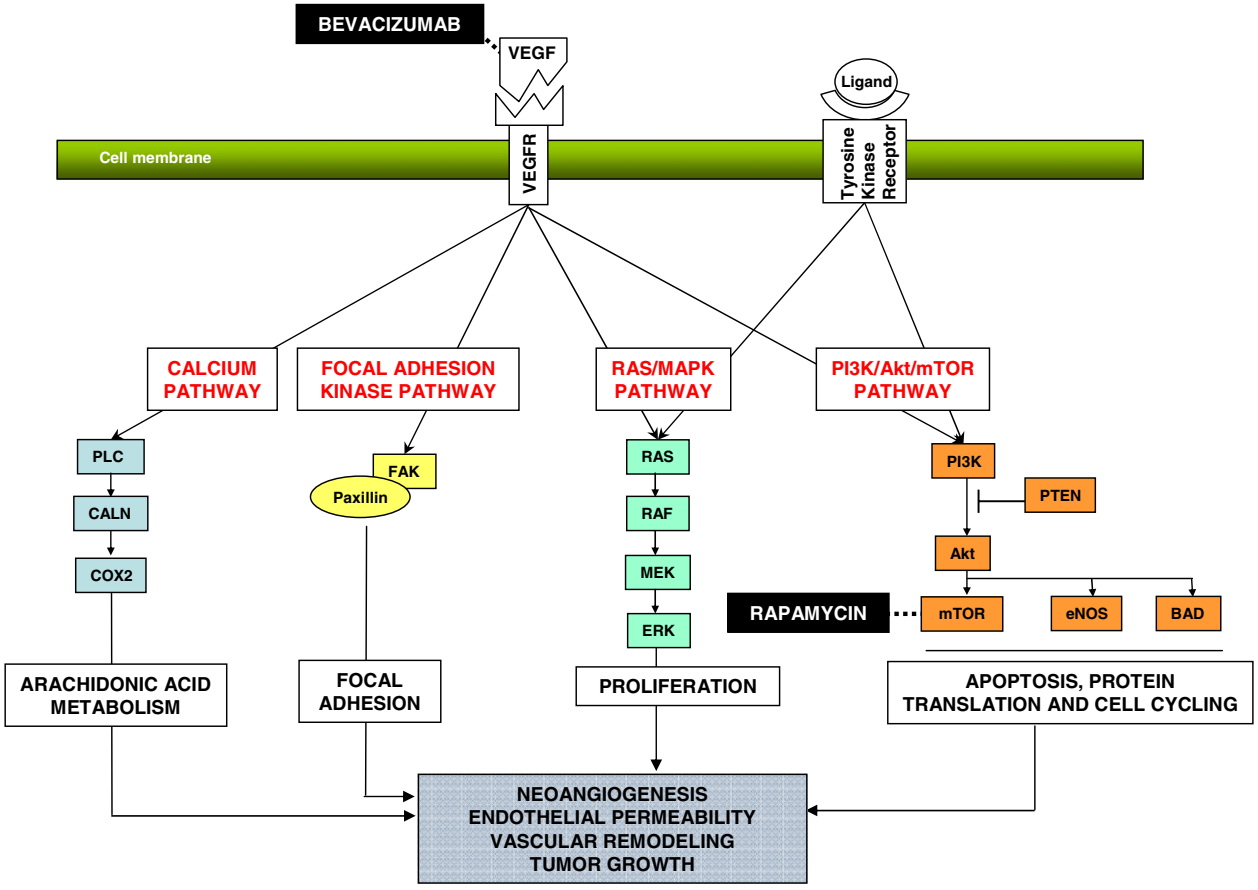

Fig. 1. Molecular targets of bevacizumab and rapamycin. Bevacizumab is a monoclonal antibody against VEGF, a ligand implicated in the pro-angiogenic response in human malignancies. Rapamycin is a small molecule blocking mTOR, an important downstream molecule of the Akt pathway. MTOR has been implicated in protein synthesis, cell growth and transition from G1 to S phase of the cell cycle. It can be activated through various growth factor tyrosine kinase receptors.

animal HCC culture models (Fig. 1). The first is a typical xenograft model in which the authors test 4 different HCC cell lines and 2 immortalized cirrhotic cell lines. This allows them to compare the sensitivity of the cancer cells and the non-cancer cells to the combined treatment, relative to the control and monotherapy treated groups. More importantly, they demonstrate that certain cell lines with oncogenic mutations are more susceptible to the drugs blocking the activated pathways than other cell lines, and describe an additive effect of the combination in tumor growth inhibition. The second model assesses the ability of cells to implant and metastasize to liver after intraperitoneal injection whilst undergoing the treatment regimens in question. Here the authors demonstrate a significant survival benefit of the bevacizumab/rapamycin combination, as opposed to the control and monotreatment groups. This experiment represents a useful departure from ectopic xenograft models in which metastases are rare. Overall, this study improves upon the routine xenograft model and demonstrates quite convincingly that the combination of the two therapies could merit further investigation in clinical trials.

Novel models are emerging to test new drugs. One solution to the disparity between cancer cell lines and human tumors is surgical orthotopic implantation, in which intact fragments of human cancer taken directly from a patient are transplanted into the corresponding organ of immunodeficient rodents, as reviewed elsewhere [18]. Another alternative is to test new drugs in xenograft models generated from cultured cancer stem cells, the key target cells to assess efficacious drugs. Further possibilities include the use of mouse cell lines in immunocompetent mice with underlying liver fibrosis [19], a model that provides a unique tool for testing efficacy of drug combinations within the context of liver fibrosis, not likely possible in immune deficient mice. Finally, a more ambitious approach would be to test novel drugs in genetically engineered mice recapitulating specific pathway abnormalities (such as double transgenic TGF/c-MYC [20], transgenic of PDGFR [21], or transgenic for β-catenin [22]) in animals with an underlying fibrotic milieu. None of the latest models are currently ready for the conventional experimental studies [16].

3. Correlation between experimental findings and clinical trials

The validity of xenografts as a predictive indicator of probable clinical activity is limited, with the most success seen in cytotoxic agents [17]. A retrospective analysis performed by the NCI for 39 compounds in which both xenograft testing and phase II clinical data were available showed that *in vivo* activity in a particular tumor histology did not closely correlate with activity in the same human cancer, and that less than 50% of agents with activity in more than one-third of xenografts showed clinical activity [23]. A similar study from the NCI of Canada comparing drug activity in phase II clinical trials, human xenograft and mouse allografts showed that the human xenograft model was predictive for non-small cell lung cancer and ovarian cancers when panels of xenografts were used, but that these same models were not predictive for breast and colon cancers [24]. More recent reviews emphasize the predictive nature of the xenograft models when pharmacokinetically clinically equivalent drug doses are tested [25].

Targeted drugs tested in pre-clinical studies and the subsequent data in clinical trials in HCC are summarized in Table 1 [26–46]. The only positive survival data reported with molecular therapies with sorafenib in HCC were preceded by strong positive pre-clinical experiments including evaluation in xenografts [27]. The remaining drugs with positive pre-clinical data have only been tested in the setting of small phase II studies, and thus the correlation between pre-clinical data and final clinical benefit (only coming from phase III studies) is difficult to predict. Although all drugs listed in Table 1 demonstrated pre-clinicial positive results, only some of them are likely to move forward according to phase II data (combinations with

Table 1
Targeted agents in pre-clinical models in HCC and clinical trial outcomes

Target	Cancer	Agent or combination	Pre-clinical studies with (+) outcomes	Clinical trial outcomes in HCC
VEGFR/Raf	HCC RCC Breast	Sorafenib	[26–28]	Phase III: survival benefit [29]
VEGFR/PDGFR	HCC	Sunitinib	[30,31]	Phase II [32,33] – survival: 11.6mo, TTR: 4.1mo
mTOR	Gallbladder, ovarian, breast	Rapamycin and its analogs everolimus, temsirolimus	[34–36]	No data in HCC. RCC phase III: survival benefit [37]
EGFR	HCC	Erlotinib	[38]	Phase II [39,40]: Survival: 13mo; TTR: 3.2mo
EGFR	HCC	Gefitinib	[41]	Phase II [42]: negative
EGFR	HCC	Cetuximab	[43]	Phase II [32,44]: negative
VEGF	HCC	Bevacizumab	No data	Phase II [30,45,46]: TTR: 6.5mo
VEGF and EGFR	HCC	Bevacizumab and Erlotinib	No data	Phase II [40]: survival: 19mo

sorafenib, bevacizumab, erlotinib and rapamycin analogs), whereas others have shown limited results (gefitinib, cetuximab and bortezomib). Several variables may impact on the divergent outcomes compared to human disease. These include degree of heterogeneity of tumors in humans versus in cell lines; the molecular aberrations of the cell line chosen, ectopic versus orthotopic location of tumor, dosage and scheduling of the two compounds, and variability in selected endpoints [47]. The greatest discrepancies between success of cancer therapies in xenograft models and in human clinical trials are likely due to critical differences in both the tumor cells and their microenvironment; this is a particularly relevant to HCC, which arises in an environment of inflammation and fibrosis.

In conclusion, as in other malignancies, we are in dire need of accurate pre-clinical models of HCC that allow us to choose which molecularly targeted therapies and combinations thereof to advance to clinical trials. However, HCC is unique in two important ways: in the heterogeneity of the tumors amongst individuals and in the microenvironment of cirrhosis in the vast majority of affected patients. The paper by Huynh et al. addresses the first need by employing a number of cell lines with known mutations and dysregulated signaling pathways. It also addresses the need for testing in a metastatic model, although a model in which the metastatic disease burden was pre-established would more accurately mimic advanced HCC in humans [25].

In order to truly justify translation of a combination therapy study into clinical trials, strong pre-clinical support is essential. The best model to test these new compounds has not yet been defined in HCC, although some novel approaches are being proposed. In parallel, serum or tissue biomarkers of molecular signatures from tumors in humans should be obtained in early trials to understand their tumor biology [2], as was recently recommended by the panel of experts in trial design in HCC [48].

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