Published in final edited form as:
Cancer Cell. 2014 May 12; 25(5): 560–562. doi:10.1016/j.ccr.2014.04.019.

Focal gains of *VEGFA*: candidate predictors of sorafenib response in Hepatocellular Carcinoma

Josep M Llovet[1,2,3]

[1]Mount Sinai Liver Cancer Program, Division of Liver Diseases, Mount Sinai School of Medicine, New York, NY 10029, USA

[2]HCC Translational Research Lab, BCLC Group, Liver Unit, IDIBAPS, CIBERehd, Hospital Clínic, University of Barcelona. Barcelona 08036, Catalonia, Spain

[3]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Catalonia, Spain

Abstract

Focal amplifications in 6p21 containing the *VEGFA* locus occur in 7-10% of hepatocellular carcinoma (HCC). A recent paper describes how VEGF-A stimulates paracrine secretion of hepatocyte growth factor by stromal cells, which induces tumor progression. HCC patients with *VEGFA* amplification are distinctly sensitive to sorafenib.

Liver cancer is a major health problem and the second cause of cancer death after lung cancer. Hepatocellular carcinoma (HCC) develops in patients with underlying chronic liver inflammation related to viral infection, alcohol, or metabolic syndrome. Sorafenib remains the only approved systemic drug for patients at advanced stages of the disease (Llovet et al., 2008). Molecular therapies targeting signaling cascades involved in hepatocarcinogenesis have been explored in phase III clinical trials, but none of the drugs tested showed positive results in first- (brivanib, sunitinib, erlotinib, and linifanib) or second-line (brivanib and everolimus) after progression on sorafenib (Llovet et al., 2014). Thus, there is an urgent need to identify molecular subclasses of HCC driven by specific genetic aberrations which can be effectively targeted recapitulating the success of crizotininb in *ALK*-rearranged lung cancer (Kwak et al., 2010) or vemurafenib in *BRAF*-mutant melanoma.

Recent studies have provided a broad picture of the mutational profile in HCC and identified an average of 30–40 mutations per tumor, among which 6-8 might be drivers (Villanueva et al., 2014, Guichard et al., 2012). Common mutations are described in the *TERT* promoter, *TP53*, *CTNNB1*, *ARID1A*, and *AXIN1*. Deep-sequencing studies confirmed frequent *TP53* and *CTNNB1* mutations in HCC and pointed to novel HCC-associated mutations in genes

Address for correspondence: Josep M Llovet, MD, Mount Sinai Liver Cancer Program, Division of Liver Diseases, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai. Madison Ave 1425. 11F-70. Box 1123, New York, NY10029. USA, Phone: 212-6599503, FAX: 212-849-2574, Josep.Llovet@mssm.edu.

involved in chromatin remodeling (*ARID1A* and *ARID2*), ubiquitination (*KEAP1*), RAS/MAPK signaling (*RPS6KA3*), oxidative stress (*NFE2L2*), and the JAK/STAT pathway (*JAK1*) (Villanueva et al., 2014, Guichard et al., 2012) (Table 1).

Studies assessing copy number alterations in HCC have consistently identified high-level amplifications at 5-10% prevalence containing oncogenes in 11q13 and 6p21, whereas other more common gains reported contain *MYC* and *MET* (Villanueva et al., 2014, Guichard et al., 2012, Chiang et al., 2008). We described amplifications of 11q13 in 5-10% of tumors, pointing to several candidate oncogenes including *CCND1* (Chiang et al., 2008). Subsequently, both *CCND1* and *FGF19* were identified in experimental models as *bona fide* oncogenes in HCC and potential targets for therapy (Sawey et al., 2011). This finding prompted the design of proof-of-concept trials testing FGFR4 inhibitors in patients with 11q13 focal amplification containing *FGF19*. Similarly, we defined high-level gains (>4 copies) of 6p21 containing *VEGFA* in 8% of cases out of 210 HCC patients explored. Interestingly, there was a significant correlation between 6p21 gains and VEGF-A mRNA expression (Chiang et al., 2008). Now an elegant study by Pikarsky's group (Horwitz et al., 2014) demonstrated that a subset of mouse and human HCCs harbors *VEGFA/Vegfa* genomic amplifications. They explored the unique role of paracrine interactions by which VEGF-A overexpression in HCC cells leads to production of hepatocyte growth factor (HGF) by stroma that reciprocally induces cancer cell proliferation. Interestingly, VEGF-A inhibition in experimental models induced HGF downregulation and patients with *VEGFA* amplification responded better to the multi-kinase inhibitor sorafenib.

Horwitz et al. found that 14% of HCC tumors developing in a mouse model of inflammation-driven cancer (MDR2-deficient mice) harbored an amplicon in a region syntenic to the human 6p21 region (Chiang et al., 2008). They confirmed *VEGFA* amplifications and/or chromosome 6 polysomy in 11% of human HCC (out of 187 cases tested). In experimental models, there was a correlation between *VEGFA/Vegfa* gains and expression levels (mRNA and protein).

To elucidate the mechanism by which *VEGFA* amplifications induced tumor progression, the authors first demonstrated that animals with amplification showed a higher vessel density, macrophage content, and enrichment for tumor-associated macrophages expression signatures. A relevant finding of the investigation is macrophage-tumor cell crosstalk. *VEGFA* amplified tumors showed higher mRNA levels, and non-neoplastic stromal cells in the microenvironment had positive HGF immunostaining. Using cells isolated from the experimental HCC models, the authors demonstrated that hepatocytes overexpressed c-Met and that macrophages overexpressed VEGFRs. In vivo studies showed that VEGF-A overexpression in HCC cells induced upregulation of HGF, mostly in macrophages, and led to increase proliferation and pro-angiogenic features. Functional confirmation of the role of VEGF-A was obtained by blocking it in MDR2-deficient mice and Hep3B xenograft models. A short course of sorafenib treatment in animals with *VEGFA* focal gains resulted in VEGF-A inhibition, decreased HGF levels, and an associated decrease in HCC proliferation. This finding is consistent with the decrease in HGF plasma levels observed in HCC patients undergoing sorafenib treatment (Llovet et al., 2012). Finally, the authors retrospectively explored a cohort of HCC patients undergoing resection who were further treated with

sorafenib. FISH-based selection of focal *VEGFA* gains defined a group of patients with better outcome, pointing to this biomarker as a potential predictor of sorafenib response.

Taken all together, Horwitz et al.'s paper provides experimental confirmation that VEGF-A has oncogenic properties in hepatocarcinogenesis by inducing paracrine secretion of HGF in stromal cells, specifically macrophages, which in turn lead to cancer cell proliferation. Notably, it would have been relevant to explore if a genetically-engineered mouse model reproducing this genomic aberration in a tissue-specific manner would have been able to recapitulate this result. Similarly, although the outcome of patients bearing *VEGFA* amplification suggests that they have a better response to sorafenib, the retrospective nature of the results precludes any firm conclusion. However, they nicely point toward further steps in designing prospective and poof-of-concept trials to confirm the hypothesis.

HCC is in need of additional molecular treatments in first- and second-line therapy and in the adjuvant setting. Reasons for recent trial failures are heterogeneous and include a lack of understanding of critical drivers of tumor progression and dissemination, liver toxicity, flaws in trial design, or marginal antitumoral potency (Llovet et al., 2014). Ongoing trials testing drugs head-to-head against sorafenib in all-comers might have difficulties in achieving superior results in first-line. Novel trials are currently designed to test drugs in biomarker-based HCC patient subpopulations. In this regard, the consequences of Horwitz et al.'s study are two-fold. First, whether *VEGFA*-amplified tumors represent a specific HCC subclass that responds better to sorafenib requires prospective confirmation with optimal control for confounding/concurring factors. Phase III trials testing sorafenib have proven benefit in all subgroup analysis of patients with advanced tumors (Bruix et al., 2012). Thus, the question to be explored is whether *VEGFA* amplified tumors certainly respond even better to this drug. Interestingly, a recent large phase III study testing sorafenib in the adjuvant setting did not meet the primary end-point of recurrence-free survival. Whether a subgroup of patients with *VEGFA* amplification might benefit from this drug in the adjuvant setting can be now elucidated. Second, high-level *VEGFA*-amplification can be used as a biomarker in phase II pivotal proof-of–concept studies testing drugs blocking VEGF-A or VEGFR2 receptors. Other drugs beyond sorafenib, such as ramurafenib (a monoclonal antibody against VEGFR2) or bevacizumab can be explored. In addition, dual VEGF-A and c-Met inhibitors appear appealing in this setting. Such inhibitors (e.g. cabozantinib) are being tested in phase III trials for second-line therapy.

We are facing a new era for testing drugs in HCC as a consequence of discovering novel oncogenic drivers (see Table 1). Although non-specific drugs will still be explored targeting all patients, pivotal proof-of-concept trials or those with biomarker-based enrichment will emerge for specific pockets of HCC patients which can completely change the treatment paradigm. The study by Pikarsky's group provides relevant information for moving towards this direction (Horwitz et al., 2014).

Acknowledgments

Josep M. Llovet has grants from National Institute of Health -NIDDK 1R01DK076986-01, National Institute of Health (Spain) grant I+D Program (SAF-2010-16055), Samuel Waxman Cancer Research Foundation, the

European Commission Framework Programme 7 (Heptromic, proposal number 259744), and the Asociación Española para el Estudio del Cáncer.

References

Bruix J, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, et al. J Hepatol. 2012; 57:821–829. [PubMed: 22727733]

Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, et al. Cancer Research. 2008; 68:6779–88. [PubMed: 18701503]

Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, et al. Nat Genet. 2012; 44:694–698. [PubMed: 22561517]

Horwitz E, Stein I, Andreozzi M, Nemeth J, Shoham A, Pappo O, Schweitzer N, Tornillo L, Kanarek N, Quagliata L, et al. Cancer Discov. 2014 [Epub ahead of print].

Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Jänne PA, Costa DB, et al. N Engl J Med. 2010; 363:1693–1703. [PubMed: 20979469]

Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, et al. N Engl J Med. 2008; 359:378–390. [PubMed: 18650514]

Llovet JM, Pena C, Lathia C, Shan M, Meinhardt G, Bruix J. Clin Cancer Res. 2012; 18:2290–2300. [PubMed: 22374331]

Llovet JM, Hernandez-Gea V. Clin Cancer Res. 2014; 20:2072–2079. [PubMed: 24589894]

Sawey ET, Chanrion M, Cai C, Wu G, Zhang J, Zender L, Zhao A, Busuttil RW, Yee H, Stein L, et al. Cancer Cell. 2011; 19:347–358. [PubMed: 21397858]

Villanueva A, Llovet JM. Nat Rev Clin Oncol. 2014; 11:73–74. [PubMed: 24395088]

Table 1

Landscape of the most prevalent mutations and high-level gene amplifications in human hepatocellular carcinoma (modified from Llovet et al., 2014).

Gene	Pathways/gene functions involved	Estimated frequency based on deep-sequencing studies (%)
Driver genes frequently mutated in HCC		
TERT promoter	Telomere stability	60
TP53	Genome integrity	20–30
CTNNB1	WNT signaling	15–25
ARID1A	Chromatin remodeling	10-16
TTN	Chromosome segregation	4-10
NFE2L2	Oxidative stress	6–10
JAK1	JAK/STAT signaling	0-9
Oncogenes/tumor suppressors rarely mutated in HCC		
IDH1, IDH2	NAPDH metabolism	<5
EGFR	Growth factor signaling	<5
BRAF	RAS/MAPK signaling	<5
KRAS, NRAS	RAS/MAPK signaling	<5
PIK3CA	AKT signaling	<5
PTEN	AKT signaling	<5
Oncogenes contained in high-level amplifications in HCC		
FGF19	FGF signaling	5-10
CCND1	Cell cycle	5-10
VEGFA	HGF signaling/ angiogenesis	7-10