Published in final edited form as:

J Hepatol. 2009 May ; 50(5): 1062–1064. doi:10.1016/j.jhep.2009.02.007.

Transcriptome analysis of liver cancer: Ready for the clinic?

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Abstract

BACKGROUND—It is a challenge to identify patients who, after undergoing potentially curative treatment for hepatocellular carcinoma, are at greatest risk for recurrence. Such high-risk patients could receive novel interventional measures. An obstacle to the development of genome-based predictors of outcome in patients with hepatocellular carcinoma has been the lack of a means to carry out genomewide expression profiling of fixed, as opposed to frozen, tissue.

METHODS—We aimed to demonstrate the feasibility of gene-expression profiling of more than 6000 human genes in formalin-fixed, paraffin-embedded tissues. We applied the method to tissues from 307 patients with hepatocellular carcinoma, from four series of patients, to discover and validate a gene-expression signature associated with survival.

RESULTS—The expression-profiling method for formalin-fixed, paraffin-embedded tissue was highly effective: samples from 90% of the patients yielded data of high quality, including samples that had been archived for more than 24 years. Gene-expression profiles of tumor tissue failed to yield a significant association with survival. In contrast, profiles of the surrounding nontumoral liver tissue were highly correlated with survival in a training set of tissue samples from 82 Japanese patients, and the signature was validated in tissues from an independent group of 225 patients from the United States and Europe ($P=0.04$).

CONCLUSIONS—We have demonstrated the feasibility of genomewide expression profiling of formalin-fixed, paraffin-embedded tissues and have shown that a reproducible gene-expression signature correlated with survival is present in liver tissue adjacent to the tumor in patients with hepatocellular carcinoma.

Hepatocellular carcinoma (HCC) is the third most deadly and fifth most common cancer in the world with about 50% of cases occurring in China. Risk factors include gender, hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, metabolism diseases, diabetes, obesity, toxins, excess alcohol consumption and smoking. HCC arises most frequently in association with inflammation due to viral hepatitis which causes over 80% of HCC cases worldwide [1].

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Gene expression in fixed tissues and outcome in hepatocellular carcinoma. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, Wrobel MJ, Lerner J, Reich M, Chan JA, Glickman JN, Ikeda K, Hashimoto M, Watanabe G, Daidone MG, Roayaie S, Schwartz M, Thung S, Salvesen HB, Gabriel S, Mazzaferro V, Bruix J, Friedman SL, Kumada H, Llovet JM, Golub TR.

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Currently, survival remains dismal for most HCC patients diagnosed with disease at advanced stages. The clinical presentation of HCC is often heterogeneous and its diagnostic and prognostic assessment is also complicated by both tumor grade and compromised liver function due to viral hepatitis, making it difficult to provide accurate clinical recommendations. Currently only about 10–20% patients are eligible for potentially curative therapies such as resection and liver transplantation. However, post-surgical survival is again complicated by relapse since most patients eventually suffer from tumor recurrence [2]. Thus, the current clinical status of HCC emphasizes the importance of defining tumor biology and the development of new screening and treatment stratification programs to refine diagnosis and improve patient outcome.

Early diagnosis is an important factor to achieve a favorable outcome since surgery can be effective in HCC patients with small tumors [2]. Therefore, an improvement of a long-term survival for HCC patients may depend on early detection. The current screening protocol for individuals at a high risk to develop HCC involves ultrasonography and detecting serum alpha-fetoprotein. However, opinions about the cost-effectiveness of the current surveillance program improving patient survival are mixed [3,4]. Analyses of over 3000 cases with small tumors (<5cm in diameter) that underwent curative resection in Liver Cancer Institute of Shanghai revealed a five-year survival in about 60% patients. However, no further survival improvement was observed in this current cohort when compared to resection of similar cases decades earlier (Tang ZY, personal communication). These findings indicate that tumor biology is an important factor in determining tumor's aggression, i.e. a small tumor detected early could have the same/similar aggressiveness as a large metastatic tumor [5]. While tumor recurrence is generally a major factor determining long-term survival of patients, the survival prognosis of an HCC patient is a complex issue. Since the majority of HCC patients suffer from two diseases, i.e., tumor and chronic fibrotic/cirrhotic liver disease, both tumor burden and diminishing liver function could independently impact patient's survival. In addition, recurrence can be further complicated by either the capacity of the tumor to metastasize or the ability of an at-risk liver to develop a second primary tumor. The unique biological characteristics of the tumor and its surrounding microenvironment are expected to reflect these situations. Therefore, a major current clinical challenge is whether we could develop a means to identify and define the biological/oncogenic characteristics of the tumor and tumor microenvironment, and select the patients accordingly for the most appropriate treatments. Global genome interrogation by the current state-of-the-art technologies promises to achieve this goal as several recent studies have begun to tackle this problem [6,7].

In their very recent work [8], Hoshida and colleagues have provided new insights into genome-based predictors of outcome in HCC patients. Using a modified cDNA-mediated annealing selection extension and ligation (DASL) assay to interrogate ~6000 genes expressed in human tumors and normal tissues including liver derived from a bioinformatic meta-analysis of public array databases, they carefully profiled HCC and non-tumor tissues from 307 patients. By partitioning samples into training and validation sets, they were able to develop a 186-gene signature of non-tumor tissues to predict HCC survival. However, Hoshida et al failed to identify any survival-associated genes when profiling tumor tissues. The most exciting and key finding of this study is the ability to globally profile gene expression from formalin-fixed, paraffin-embedded (FFPE) tissues and to identify a surrounding non-tumoral liver tissue-associated gene signature predictive of HCC survival. This is significant since a vast majority of clinical specimens available in clinics are FFPE samples. In contrast to frozen tissues, FFPE tissues can be easily archived and transported, and thus can readily facilitate in discovery and validation of genome-based predictors for stratification of patients into homogeneous classes for survival, recurrence, etiology, oncogenic pathways etc. Although the new findings are encouraging, the future challenge remains how one can effectively and reliably translate this technology into clinical practice. Platform stability, the robustness of prediction algorithm and

additional large independent cohort from multi-site, blinded validation study, such as those in lung adenocarcinoma [9], remain to be further explored.

Profiling FFPE tissues by a candidate-gene profiling approach using quantitative reverse-transcriptase-polymerase-chain-reaction (qRT-PCR) has been successful in the past [10]. However, a genomewide expression profiling of FFPE tissues is still a challenging task. This study reported a successful story about profiling ~6000 transcripts in a large cohort of HCC cases. It is debatable whether the use of these 'transcriptionally informative genes' can be considered an unbiased global approach. A requirement is that these transcripts should be detectable by microarray from a bioinformatic meta-analysis of publicly available gene expression datasets, which include 1711 tumors and 438 non-tumor samples. It is possible that these preselected genes represent genes with a relatively high abundance. A significant amount of important genes are often silenced in non-tumor tissues (in this case surrounding nontumoral liver tissue) and in tumor tissues either through epigenetic silencing or somatic mutations, and many tumor-related genes can often express in low abundance. Obviously, these genes would be excluded from the study. This could be the explanation why this report fails to identify tumor-associated survival genes. Therefore, caution should be exercised in the interpretation of any biological activities by performing pathway analysis from the identified gene set. Encouragingly, the whole-genome DASL assay is now available and can thus be used to address these issues.

As discussed above, recurrence can be the result of metastasis and/or the development of a *de novo* tumor. Distinct biological activities contributed by both tumor and/or hepatic microenvironments are expected to distinguish these two scenarios. The reported study by profiling non-tumor samples to predict late recurrence may address the latter scenario. The use of gene expression signature in non-tumor samples to predict metastasis and early recurrence of HCC patients was first reported by Budhu et al [7]. The current study is consistent with the previous finding that non-tumor-derived molecular signatures can predict outcomes. However, the identified signature by Budhu et al may be mainly useful for predicting metastasis-related recurrence (i.e., early recurrence) while the current signature may be predictive of risk for the development of *de novo* tumor (i.e., late recurrence). Consistent with this notion, a comparison of the two gene signatures (186 vs 454 genes) reveals only one overlapping gene (GHR). Similarly, early and late recurrence-related genome-predictors are expected to exist in tumors [11]. Thus, it is possible that there are at least four different signatures to be anticipated, two from tumor and two from non-tumor tissues, which could be useful to predict outcomes. The notions that advanced tumors are only restricted to those identified in a late stage and that late recurrence is typical of small tumors are conceptually and clinically misleading. Such an argument would ignore the fact that many small tumors could be equally aggressive to those found at late stages and many large tumors could be equally share the low level of aggressiveness observed in small tumors [5]. It is noted in the current study that many cases have an early recurrence. Consistent with other published data, reanalyzing the clinical data recently published from 169 solitary small tumors (<5cm) [12] reveals that among 78 cases with recurrence, >60% had early recurrence. Thus, early recurrence is still a major clinical problem. This has important conceptual and clinical implications in our pursuits for how best to stratify and treat HCC patients effectively. HCC heterogeneity should not be understated as it is critical to achieve an accurate prognosis prediction and patient stratification for an effective treatment. It is increasingly recognized that the underlying molecular activities are far superior to phenotypic surrogate parameters, such as age, gender, tumor size, cirrhosis in defining patient outcomes. There is a clear need to profile patients' outcomes based on an unbiased approach and genomics technologies offer superior tools to archive this goal.

Acknowledgments

This work was supported by the Intramural Research Program of the Center for Cancer Research, the US National Cancer Institute, the National Institutes of Health.

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