Gene Expression in Hepatocellular Carcinoma: Pilot Study of Potential Transarterial Chemoembolization Response Biomarkers

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ABSTRACT

Purpose: To perform a feasibility study to explore the relationship between hepatocellular carcinoma genetics and transarterial chemoembolization treatment response to identify potential biomarkers associated with enhanced treatment efficacy.

Materials and Methods: In this single-institution study, pretreatment hepatocellular carcinoma biopsy specimens for tumors in 19 patients (14 men, five women; mean age, 59 y) treated with chemoembolization between 2007 and 2013 were analyzed for a panel of 60 chemotherapy-sensitivity, hypoxia, mitosis, and inflammatory genes with the QuantiGene Plex 2.0 mRNA detection assay. Demographic, disease, and procedure data and tumor response outcomes were collected. Quantitative mRNA levels were compared based on radiologic response between tumors exhibiting complete response (CR) versus partial response (PR).

Results: The study sample included 19 biopsy specimens from tumors (mean size, 3.0 cm; grade 1, n = 6; grade 2, n = 9; grade 3, n = 4) in patients treated with a mean of two conventional chemoembolization sessions. Thirteen and six tumors exhibited CR and PR, respectively, at a mean of 116 days after treatment. Tumors with CR showed a significant increase in ($P < .05$) or trend toward ($P < .1$) greater (range, 1.49–3.50 fold) pretreatment chemotherapy-sensitivity and mitosis (*ATF4*, *BAX*, *CCNE1*, *KIF11*, *NFX1*, *PPP3CA*, *SNX1*, *TOP2A*, and *TOP2B*) gene mRNA expression compared with tumors with PR, in addition to lower *CXCL10* levels (0.48-fold), and had significantly ($P < .05$) higher (1.65-fold) baseline *VEGFA* levels.

Conclusions: Genetic signatures may allow prechemoembolization stratification of tumor response probability, and gene analysis may therefore offer an opportunity to personalize locoregional therapy by enhancing treatment modality allocation. Further corroboration of identified markers and exploration of their respective predictive capacity thresholds is necessary.

ABBREVIATIONS

CR = complete response, FFPE = formalin-fixed, paraffin-embedded, HCC = hepatocellular carcinoma, qPCR = quantitative polymerase chain reaction, PR = partial response

Transarterial chemoembolization is widely used in the contemporary management of hepatocellular carcinoma (HCC) (1). Although objective tumor response rates after chemoembolization are generally favorable and may exceed 60%–80% (2,3), viable tumor foci can nonetheless remain present in approximately 30%–60% of cases (2–4); such treatment failure has been predominantly attributed to chemotherapy resistance and ischemia-induced angiogenesis spurring tumor growth (5). Resistance may affect the efficacy of several cytotoxic agents (6), and hypoxia has been shown to result in upregulation of vascular growth factors (7,8) and increase in tumor microvessel density (8,9), a factor associated with tumor recurrence (10). Although chemotherapy resistance and tumor hypoxia have therefore been linked with tumor progression, an understanding of the fundamental influence of these effects on chemoembolization treatment

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outcome is undetermined, and represents a major gap in knowledge on the topic of chemoembolization. Although current histologic and radiologic techniques ably detect and classify tumor response to therapy after transcatheter treatment (3), these methods are incapable of providing insight into the primary biologic mechanisms that may predispose to treatment success or failure.

Tumor genetic analysis represents an encouraging contemporary approach to improve understanding of the principal mechanisms that underlie therapeutic outcome after cancer treatment (11). Gene expression has been previously applied to fixed tumor tissues to discover a reproducible gene-expression signature associated with improved overall HCC survival (12). Similarly, genetic analyses assessing HCC chemotherapy sensitivity (13) and response to hypoxia (14) have identified genetic monikers associated with cytotoxic and ischemic tissue insults. Given these past successes, it is plausible that tumor genetic analysis may offer the potential to identify innate tumoral factors associated with favorable or adverse outcomes after interventional oncologic locoregional transarterial therapy. Yet, investigation of the association between tumor genetics and transarterial chemoembolization has not been extensively explored to date. The present study was therefore undertaken with the intent of characterizing a chemoembolization-relevant HCC genetic profile and exploring the relationship between identified genetic elements and chemoembolization treatment response to detect potential biomarkers associated with enhanced therapeutic efficacy.

MATERIALS AND METHODS

Institutional review board approval was granted for this study. Patients provided written informed consent for HCC biopsy procedures.

Patients and Tumors

Patient cohort. One hundred eighty-eight patients with HCC treated with ethiodized oil–based transarterial chemoembolization between January 2007 and December 2013 at a single tertiary-care hospital were identified via review of the department of radiology picture archiving and communications system and interventional radiology case log book. Treatment allocation to chemoembolization was determined at a multidisciplinary tumor board, and was generally offered to patients with Barcelona Clinic Liver Cancer stage B disease, although stage A or C disease was not an exclusion criterion. Inclusion criteria for chemoembolization included HCC treatment with therapeutic intent as a "bridge" or downstaging strategy before liver transplantation. Relative contraindications included total bilirubin level $>$ 3.0 mg/dL, serum creatinine level $>$ 2.0 mg/dL, International Normalized Ratio $>$ 1.5, platelet count $< 50 \times 10^3/\mu L$, and Eastern Cooperative Oncology Group performance status $\geq$ 2.

Tumor identification. The 188 patients bore 207 index tumors, defined as the largest treated tumor focus in each liver lobe. Of the 207 tumors identified, 146 (71%) were diagnosed based on established imaging criteria (15)—rendering biopsy unnecessary—and were therefore excluded. Sixty-one tumors (29%) that underwent biopsy tissue sampling were therefore eligible for study inclusion. Liver mass biopsies, performed for lesions with indeterminate imaging characteristics, were performed with ultrasound guidance, and tissue samples were immediately fixed in 10% formaldehyde solution and then embedded in paraffin and sectioned for hematoxylin and eosin staining.

Among the 61 tumors that underwent biopsy, only 38 (62%) paraffin-blocked pathologic specimens were physically available from the department of pathology and deemed satisfactory for use in the study; 23 (38%) lacked sufficient remaining tissue or were not located for analysis. Tissue adequacy entailed ample tissue to allow at least 25–100 $mm^2 \times 100\mu m$ (pooled) tissue sections as required by the genetic assay system used. Thirty-eight biopsy-proven treatment-naive HCC tissue specimens therefore constituted the study sample for descriptive genetic characterization. **Table 1** summarizes the features of the principal study cohort.

Outcomes correlation subset. The study cohort was further narrowed to investigate the relationship between HCC genetic profile and tumor radiologic response. For this analysis, 15 tumors treated with concurrent percutaneous ablative therapy, consisting of same-day or next-day radiofrequency ablation (n = 14) or percutaneous ethanol injection (n = 1), were excluded from the 38-tumor cohort because of the confounding impact of ablation on treatment outcome. Four tumors with infiltrative morphology were also omitted as a result of perceived incomparability with focal encapsulated HCC tumors, which constituted the majority of tumors. Nineteen focal encapsulated HCC nodules in patients treated with chemoembolization therefore constituted the subset for exploration of genetic factors associated with tumor response. **Table 1** also presents the features of this subset population.

Transarterial Chemoembolization Procedures

The 36 patients in the study cohort underwent a mean of two (standard deviation, 1) selective or lobar chemoembolization sessions, performed by two Certificate of Added Qualifications–licensed interventional radiologists with 10 and 6 years of experience, respectively. For chemoembolization procedures, a microcatheter was positioned in the lobar (n = 5 initial treatments) or