

Can Transarterial Chemoembolization of Hepatocellular Carcinoma Result in Transformation to Combined Hepatocellular-Cholangiocarcinoma with Stem Cell Features? A Case Study

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Transarterial chemoembolization (TACE) is currently one of the favored treatment modalities for non-curative hepatocellular carcinoma (HCC) and can be used to shrink tumor size in order to make a patient eligible for transplantation. Furthermore, with the advent of effective antiviral drugs for hepatitis B virus (HBV), concomitant antiviral therapy with local tumor ablation including TACE for HBV-associated HCC has been successful for long term survival. Over the last decade, however, concern has been raised about a phenomenon whereby a subset of TACE-treated HCCs becomes more aggressive after TACE treatment. One current hypothesis is that TACE eliminates only the hepatocellular cells and that hepatic progenitor cells that have the potential for developing to cholangiocarcinoma are then selected for and induced to proliferate post-TACE with possible dual differentiation along hepatocellular and biliary lines. We present a case of a patient with HCC who underwent TACE and subsequently experienced tumor regrowth as biopsy-proven combined hepatocellular-cholangiocarcinoma. Furthermore, we provide immunohistochemical evidence of hepatic progenitor cells in the post-TACE tumor biopsy, possibly accounting for its aggressive course.

[*NAJ Med Sci.* 2017;10(4):181-186. DOI: 10.7156/najms.2017.1004181]

Key Words: *transarterial chemoembolization, hepatocellular carcinoma, stem cell*

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the third cause of cancer-related mortality globally.¹ While liver transplantation is the therapy of choice, the shortage of organ supply has been one of the major difficulties for patients in need. Since effective drugs for hepatitis B virus (HBV) have become available, concomitant anti-HBV therapy with local tumor ablation or resection has demonstrated significantly improved survival of these patients, as summarized by Yuan et al.² In fact, our institution (Thomas Jefferson University Hospital) has reported the longest survival of HBV-HCC patients with the above treatment modality.³ Transarterial chemoembolization (TACE) is frequently used for local tumor ablation. It is also the favored treatment for non-curative HCC and can be used to shrink tumor size in order to make a patient eligible for

transplantation. The procedure achieves cytoreduction or eradication through both ischemic and chemotherapeutic means. TACE has shown objective response rates in 16-60% of patients, with complete response achieved in less than 2%.³ Over the last decade, however, concern has been raised about a phenomenon whereby a subset of TACE-treated HCCs becomes more aggressive after TACE treatment. One current hypothesis is that TACE eliminates only the hepatocellular cancer cells and that hepatic progenitor cells that have the potential for developing into cholangiocarcinoma are then selected for and induced to proliferate post-TACE with possible dual differentiation along hepatocellular and biliary lines. We present a case of a patient with HBV-associated HCC that underwent TACE and then experienced tumor regrowth as combined hepatocellular-cholangiocarcinoma (HCC-CC). Furthermore, we provide immunohistochemical evidence of hepatic progenitor cells (HPCs) in the post-TACE tumor biopsy, possibly accounting for its aggressive course.

Received: 08/21/2017; Revised: 10/02/2017; Accepted: 10/17/2017

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CASE REPORT

A 26 year-old Asian male was noted to be HBsAg (+) at age 26 during circumcision in 11/2005. Family history revealed his mother negative for HBsAg and anti-HBs but positive for anti-HBc. His maternal grandmother was HBsAg (+). Also his paternal great uncle had hepatitis B with cirrhosis. He was likely to have been infected during early childhood. He was HBeAg (+) with HBV DNA 2.8×10^5 copies/ml. He was started on adefovir by his hepatologist.

In 8/2006, MRI showed nodular liver with splenomegaly and paraesophageal varices. There was no tumor seen imaging. Serum albumin was 3.9 gm/dl, ALT 66 U/L, platelets $94,000/\text{mm}^3$, HBeAg (+) and HBV DNA was 1.4×10^3 copies/ml. Lamivudine was added to adefovir.

In 3/2007, MRI showed a 1.2cm HCC in the right posterior lobe (segment 7). AFP was 2.9 ng/ml. Options on various therapies including local ablation, liver transplantation, and resection were discussed and the patient opted for local ablation. He said he would rather take multiple tumor ablations than undergo transplantation. He received successful percutaneous ethanol injection. He remained tumor free for 3 years until 7/2010 when MRI showed a new 1.3 cm enhancing tumor mass in the right lobe (segment 6). AFP was 442 ng/ml with 83.6% AFP-L3 indicative of HCC.

Following discussion with the patient and his family, the patient opted for TACE. However, 10 months later (7/2011), MRI showed a 3.1 cm recurrent tumor at the treated site. On his insistence for a local procedure rather than transplantation, he underwent TACE followed by laparoscopic microwave ablation in 9/2011. AFP was 517 ng/ml with AFP-L3 84.8%. He was started on sorafenib. Sorafenib was poorly tolerated, and in 7/2012 after clinical progression he was enrolled in a Phase 2 clinical trial testing a new agent for metastatic HCC. AFP was 8344 ng/ml. At the first clinical evaluation he showed significant progression in the liver with increase in AFP to 29,373 ng/ml and liver biopsy was obtained in 10/2012. The result was combined HCC-CC.

Further immunochemical studies were carried out as described below.

METHODS

For the purpose of this study, we confirmed the dual differentiation of the tumor by reviewing the hematoxylin and eosin (H&E) stained slides of the post-TACE liver biopsy. In addition, evaluation for HPCs was done by immunohistochemical staining for EpCAM, NCAM, CK19, CK7, and hepatocyte specific antigen (HSA) on formalin-fixed, paraffin-embedded tissue (**Table 1**) using the Ventana Ultraview detection kit (Ventana Medical Systems, Tucson, AZ) with the BenchMark Ultra IHC staining module. Five micron sections were cut using a microtome. The slides were deparaffinized in a dry oven at 72°C for 20 minutes. They were then placed into a tris-based buffer with basic pH called ULTRA cell conditioner #1 (Ventana, catalog #950-224). The slides were then brought to $36-37^\circ\text{C}$ and incubated for four

minutes. Specific antibodies were then added as follows: anti EpCAM (clone BerEP4), anti-CD56 (clone 23C3mAb), anti-CK19 (clone A53-B/A2.26), anti-CK7 (clone SP52), and anti-HSA (clone OCH1E5). For staining amplification, an additional step for anti-CD56 was required where mouse antibody was added and allowed to incubate at 36°C for an additional 12 minutes. Excessive antibody was then washed off using ultraWash solution (Ventana Medical Systems). The slides were then counterstained with hematoxylin and incubated for 8 minutes. The stained slides were examined under a microscope for nuclear, cytoplasmic, and/or membranous staining of the morphologically glandular and hepatocellular components to determine positivity or negativity. The criteria for positivity were the percentage of cells positive within the lesion: $< 5\%$ = negative, $5-24\%$ = 1+, $25-49\%$ = 2+, and 50% or more = 3+ positivity. Intensity of staining was not taken into account for the purpose of this study.

RESULTS

The tumor displayed both an infiltrative glandular component in the form of tubules with lumens and moderate nuclear atypia intermixed with a well-differentiated hepatocellular component in the form of thickened plates of hepatocytes in a trabecular pattern, increased nuclear size, and prominent nucleoli (**Figure 1, A-B**). As expected, HSA antibody showed expression in the HCC component with 1+ cytoplasmic staining while the glandular component showed no positive staining (**Figure 1, C-D**). EpCAM expression was diffusely 3+ positive in the cytoplasm of the HCC component and 1+ positive in the glandular component (**Figure 1, E-F**). NCAM showed 3+ positivity in the cell membranes and cytoplasm of glandular components while the HCC component was completely negative (**Figure 2, A-B**). CK19 showed diffuse cytoplasmic staining in not only the malignant glandular component but also in the well differentiated HCC component (3+ positivity in both areas, **Figure 2, C-D**). CK7 was expressed within the cytoplasm of the tumor cells in the HCC component with 2+ positivity and in the glandular component with 3+ positivity (**Figure 2, E-F**). In summary, the post-TACE tumor biopsy showed morphology of HCC-CC and revealed expression of stem cell markers EpCam, NCAM, CK19, and CK7 by immunohistochemistry (**Table 2**).

DISCUSSION

We present a patient with HBV infection and radiologically-confirmed HCC who underwent TACE, whose post-procedure course was notable for a tumor with rapid growth and increased aggressiveness that eventually resulted in the patient's death. Biopsy of his tumor post-TACE revealed combined HCC-CC with stem cell features, including expression of immunohistochemical markers associated with hepatic progenitor cells (HPCs), specifically EpCam, NCAM, CK19, and CK7.

Combined HCC-CC is a relatively rare tumor, comprising less than 1% of all liver carcinomas.⁴ As defined by the World Health Organization, combined HCC-CC is classically defined as having areas of typical HCC and areas of typical CC

intimately mixed.⁴ If tumor cells that have a morphology or immunophenotype of stem cells/HPCs predominate, the term “combined HCC-CC with stem-cell features” is recommended.⁴ There is growing evidence that the TACE procedure may cause transformation of typical HCC to combined HCC-CC with stem cell features. No clear consensus exists on which markers to use for hepatic stem cells/HPCs. CK7 is considered a marker of intermediate hepatocytes (a committed hepatocyte precursor) and CK19 a marker of HPCs.⁵⁻⁷ Nishihara et al⁸ showed that HCC treated with TACE had significantly increased CK19 expression compared to untreated HCC. This was expanded upon by Zen et al⁹ who showed significantly increased expression by

immunohistochemistry and RT-PCR for CD133, CK19, EpCAM, and NCAM in TACE-treated tumors versus untreated tumors, as well as increased HCC-CC in TACE-treated tumors. Zeng et al¹⁰ showed similar results, with TACE-treated tumors showing significantly increased EpCAM and CD133 expression compared to untreated tumors. Importantly, the above studies excluded any cases that showed either radiologic or biopsy evidence of combined HCC-CC prior to the TACE procedure. Therefore, our case supports the growing evidence that a subset of TACE-treated tumors may transform to combined HCC-CC and the increased expression of markers associated with HPCs implicates stem cells in this process.

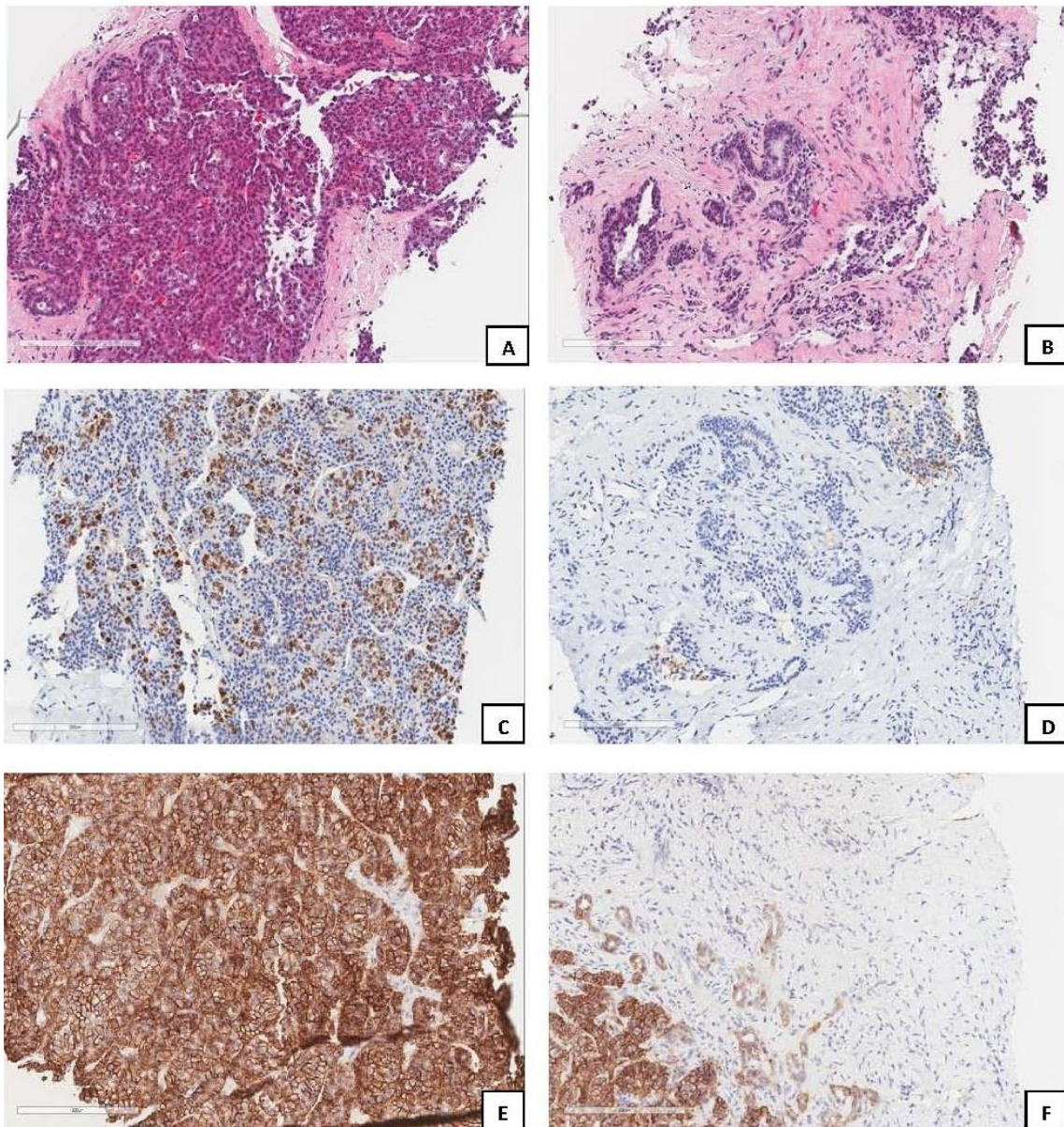


Figure 1. A) Well differentiated hepatocellular carcinoma component, H&E 200x B) Cholangiocarcinoma component, H&E 180x C) and D) HSA immunohistochemical (IHC) stain with 1+ positivity in HCC (C) and negative HSA IHC in cholangiocarcinoma component (D). E) and F) EpCAM IHC stain with 3+ positivity in HCC component (E) and 1+ positivity in cholangiocarcinoma component (F).

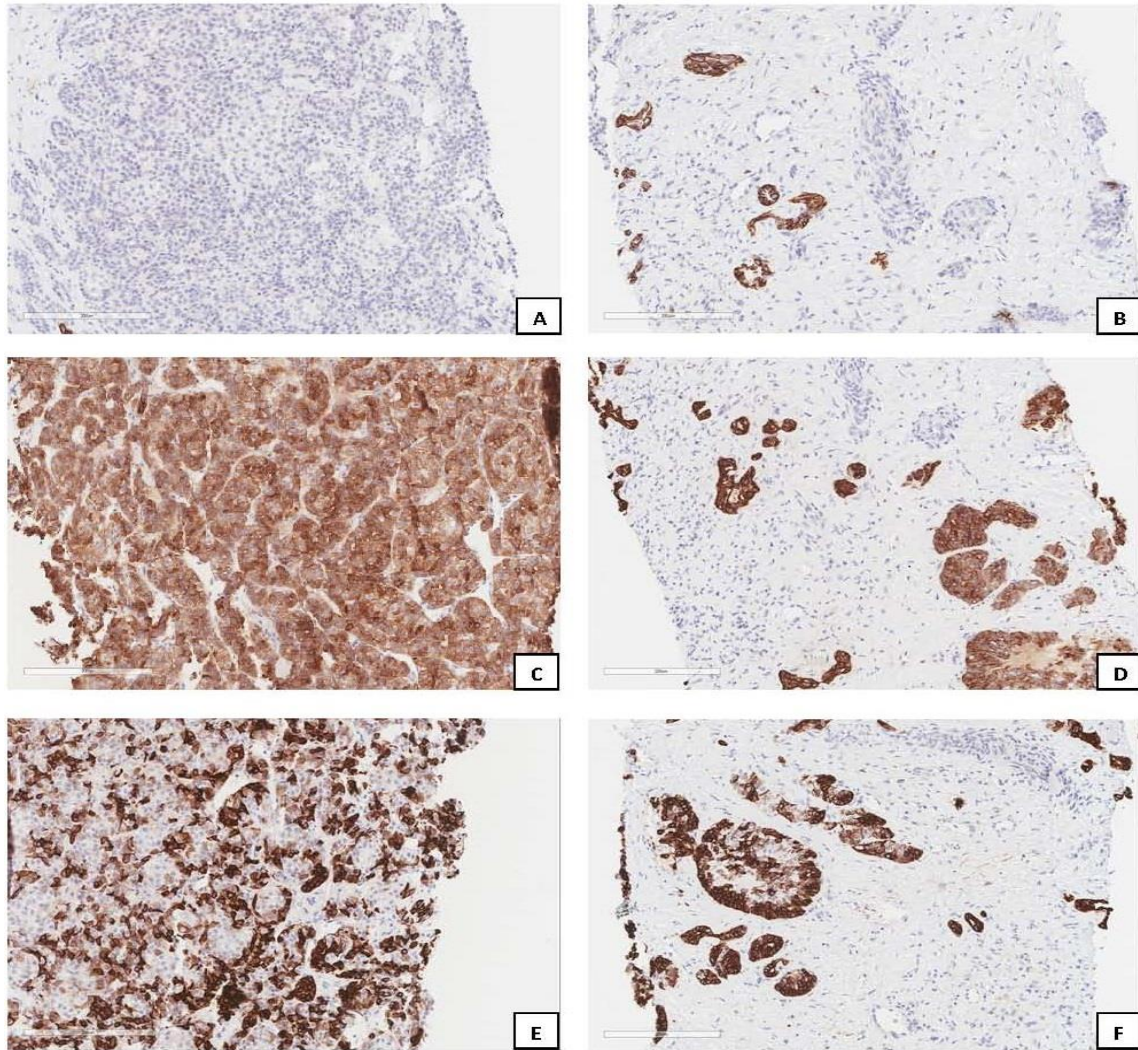


Figure 2. A) Negative NCAM IHC stain in the HCC component, 200x B) NCAM IHC stain with 3+ positivity in the cholangiocarcinoma component, 200x C) and D) CK19 immunohistochemical (IHC) stain with 3+ positivity in HCC (C) and cholangiocarcinoma component (D). E) and F) CK7 IHC stain with 2+ positivity in HCC component (E) and 3+ positivity in cholangiocarcinoma component (F).

The true significance of increased HPC marker expression in TACE-treated tumors lies in the more aggressive tumor biology. Several studies have shown that TACE-treated HCC tumors expressing CK7, CK19, CD133, and/or EpCAM - markers associated with HPCs - show significantly increased recurrence rates after transplant compared to untreated tumors.⁹⁻¹³ Why a therapy that has been shown to shrink and rarely eradicate HCC tumors should result in increased aggressiveness in a subset of patients is not currently known. Several studies have shown that HCCs treated with TACE

have residual viable tumor most of the time.¹⁴⁻¹⁷ Moreover, there is evidence of increased proliferation of both intratumoral endothelial cells and tumor cells in TACE-treated tumors compared to untreated tumors, as measured by Ki-67 immunohistochemistry.¹⁸

A current unifying theory to explain these findings suggests the residual viable tumor cells after TACE contain a population of chemotherapy- and/or ischemia-resistant HPCs that are induced to undergo proliferation after TACE.^{9,10,19}

Table 1. Immunohistochemical stains with staining patterns and cellular localization.

Immunohistochemical stain	Cellular localization	Staining pattern
Cytokeratin 7 (CK7)	cytoplasmic	Positive in hepatic stem cells, benign biliary ducts and cholangiocarcinoma; positive in HCC in only 20% of the cases
Cytokeratin 19 (CK19)	cytoplasmic	Positive in hepatic stem cells, benign biliary ducts and cholangiocarcinoma; usually negative staining in HCC
NCAM (CD56)	membranous /cytoplasmic	Positive staining in progenitor cells committed to biliary lineage and reactive biliary epithelium
EPCAM (BerEP4)	membranous	Positive in hepatic stem cells; negative in mature hepatocytes
HSA (HepPar1)	cytoplasmic	Positive in benign and malignant hepatocytic tumors-hepatoblastoma, hepatic adenoma, HCC; negative staining in cholangiocarcinoma

Table 2. Stem cell marker expression in combined HCC-cholangiocarcinoma.

Antibody	Location	HCC component	Cholangiocarcinoma component
CK7	cytoplasmic	2+	3+
CK19	cytoplasmic	3+	3+
NCAM (CD56)	membranous /cytoplasmic	Negative	3+
EpCAM	Membranous	3+	1+
HSA	cytoplasmic	1+	Negative

*The expression was considered positive or negative based on the percentage of cells that expressed the antigen in the lesion: < 5% = negative expression, 5-24% = 1+, 25-49% = 2+, ≥ 50% = 3+.

These HPCs are capable of bipotential differentiation into hepatocytic and cholangiocytic/biliary phenotypes, thus accounting for the combined tumor morphology. Therefore, a combination of treatment resistance and increased proliferation may account for the development of combined tumors with stem cell features that arise after TACE therapy with poor prognosis.

Our case highlights the potential for TACE therapy to result in an unintended consequence of increased tumor aggressiveness. The exact incidence of this occurrence is unknown but is relatively rare. Our service performs approximately 200 TACE procedures for HCC each year and less than five HCC-CC tumors have been identified in the last five years. Unfortunately, a pre-TACE biopsy of this patient's tumor was not obtained to allow comparison with the post-TACE sample; thus, we are not able to definitively show a histologic and immunophenotypic transformation. This is commonly the case in clinical practice, as HCC has diagnostic imaging characteristics that obviate the need for a tissue diagnosis.³ In the current case, the diagnosis of HCC was supported by his AFP level of 517 ng/ml with AFP- L3 84.8 %.^{20,21} Based on this observation, the possibility of transformation of HCC to CC or combined HCC-CC should be considered if TACE treated HCC becomes aggressive and poorly responsive to treatment. Future research is needed to address three questions: Is performing HPC marker immunohistochemistry on a pre-TACE biopsy useful to predict recurrence after treatment? Which patients might benefit from post-TACE tumor biopsies with HPC markers performed to assess for tumor transformation? Given the small but possible risk of tract seeding from biopsy, is there a potential role for liquid biopsy to determine tumor aggressiveness?

CONFLICT OF INTEREST

None.

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