Comparative Genomic Hybridization

Synchronous Occurrence of Focal Nodular Hyperplasia and Hepatocellular Carcinoma in the Same Liver Is Not Based on Common Chromosomal Aberrations

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Key Words: Carcinogenesis; Genetic; Chromosomes; Comparative genomic hybridization; CGH

DOI: 10.1309/EF69VNDLVWPVE4QV

Abstract

Occasionally hepatocellular carcinomas (HCCs) occur synchronously with or within focal nodular hyperplasias (FNHs), raising the question of a putative causal relationship. In the present study, we used comparative genomic hybridization to investigate the occurrence of genomic aberrations in FNHs, which might lead to hepatocarcinogenesis. Tissue samples from FNHs and nonlesional liver tissue were obtained from 7 women. None of the patients had a chronic diffuse liver disease. A synchronous HCC not spatially related to FNH was present in 1 patient. Two patients had received oral contraceptives. Genomic aberrations were found in only 1 FNH. No aberration was found in the FNH occurring synchronously with HCC, but the HCC included gains at chromosomes 1q, 5, 12, and 19q and losses at 4p, 7q22-q35, 9p, 17p, 21q, and 22q. No aberrations were found in nonneoplastic liver tissues. Our findings support the notion that FNH is not a preneoplastic lesion for the occurrence of HCC in humans and that the synchronous occurrence of FNH and HCC is coincidental in our case.

Hepatocellular carcinomas (HCCs) might evolve from precancerous lesions, such as dysplastic nodules, and, occasionally, from liver cell adenomas.1,2 Study of these lesions might facilitate understanding of hepatocarcinogenesis. Identification of candidate oncogenes and tumor suppressor genes for hepatocarcinogenesis is a major challenge and might permit identification of patients at risk and an evaluation of targets for therapeutic treatments. It generally is accepted that cancer proceeds through accumulation of mutations in genes that govern cell proliferation and death. HCCs display genomic alterations, including DNA rearrangements, loss of heterozygosity, chromosomal amplifications, loss of imprinting, and mutations.

Different genes have been implicated in the pathogenesis of HCC and may be divided into 4 major groups: (1) genes regulating DNA damage response (P53 pathway: p53), (2) genes involved in cell cycle control (retinoblastoma [RB1] pathway: RB1, p16INK4A, cyclin D), (3) genes involved in growth inhibition and apoptosis (transforming growth factor beta pathway: mannose-6-phosphate/insulin-like growth factor 2 receptor [M6P/IGF2R], SMAD2, SMAD4), and (4) genes responsible for cell-cell interaction and signal transduction (adenomatous polyposis coli [APC]/beta-catenin pathway: APC, beta-catenin, E-cadherin) (for a review see Öztürk3). Hepatocarcinogenesis could be mediated by loss of heterozygosity (RB1, M6P/IGF2R gene, E-cadherin gene, BRCA2 [breast cancer 2]), somatic mutation (p53, RB1, p16INK4A, M6P/IGF2R, SMAD2, SMAD4, beta-catenin, APC, BRCA2), de novo methylation (p16INK4A, E-cadherin gene), and/or functional inactivation (p53).

The pattern of genomic alterations shows great variability. As yet there is no evidence for an ordered sequence of
genomic events leading to hepatocarcinogenesis, and the absence of an obviously inherited predisposition to liver cancer has hampered the identification of critical genes in hepatocarcinogenesis, making synchronous liver cell tumors in a noncirrhotic liver particularly interesting for the study of tumorigenesis.

The aim of the present study was to prove by comparative genomic hybridization (CGH) analyses whether synchronous FNH and HCC in a patient without evidence of a chronic diffuse liver disease might have been related to a common genomic aberration. Data obtained in this particular case were compared with data for 6 additional FNHs obtained from patients without synchronous HCC.

**Materials and Methods**

Seven FNHs and 1 HCC from 7 patients were used in the present study. All patients underwent operation between February 1998 and February 2001. Patients’ ages ranged from 22 to 54 years (mean, 43 years; median, 48 years). The diameter of the resected tumors ranged from 1.8 to 16.0 cm (mean, 7.0 cm; median, 8.9 cm) [Table 1.](#)

One patient had both an FNH and an HCC (case 4, Table 1). Tissue samples (FNHs, HCC, and nonlesional liver tissue) for CGH were obtained immediately after surgery, snap frozen in liquid nitrogen, and stored at –80°C until further use.

**Histologic Specimen Processing**

The remaining tissue was used for routine histologic examination. Tissue samples from every tumor and nonneoplastic liver were fixed in 10% neutralized formalin and embedded in paraffin. Deparaffinized serial sections were stained using H&E, periodic acid–Schiff reagent with and without diastase pretreatment, Masson trichrome stain, reticulin stain, and iron stain. Immunostaining was performed with polyclonal antibodies directed against alpha fetoprotein (DAKO, Glostrup, Denmark) as described elsewhere.4

**DNA Preparation and CGH**

For DNA preparation, a small frozen tissue piece (average, approximately 2 mm³) was dissected using a scalpel and dissolved in lysis buffer of the QIAmp DNA Mini kit (Qiagen, Hilden, Germany). Further DNA preparation, including RNA digestion, was performed according to the manufacturer’s instructions. CGH was performed as described by Arnold et al,5 with few modifications. In brief, genomic DNA was labeled with digoxigenin-11–deoxyuridine triphosphate and biotin-16–deoxyuridine triphosphate (Roche Diagnostics, Penzberg, Germany) for reference and test DNA. Reaction conditions were optimized to yield labeled fragment sizes ranging from 200 to 2,000 base pairs. Two hundred to 300 ng of biotinylated tumor DNA was ethanol coprecipitated with the same amount of labeled control DNA and 10 to 15 µg of unlabeled human Cot-1 DNA (Gibco BRL, Life Technologies, Eggenstern, Germany). The probe mixture was dissolved in 10 µL of hybridization buffer (50% formamide, 10% dextran sulfate, 2× standard saline citrate [SSC]), denatured, and, after preannealing (30 minutes), hybridized onto normal metaphase chromosomes (Vysis, Bergisch Gladbach, Germany) for 2 to 3 days at 37°C in a moist chamber. Posthybridization washes were performed without formamide as follows: 1× phosphate-buffered detergent (PBD) (Appligene Oncor, Illkirchen, France) at room temperature for 5 minutes, 2× SSC at 70°C for 5 minutes and again 1× PBD at room temperature for 5 minutes. The DNA was detected using anti–digoxigenin–rhodamine (red) and streptavidin–fluorescein isothiocyanate conjugates (green). Slides were counterstained with 4′,6 diamidino-2-phenylindole-dihydrochloride at a concentration of 0.1 µg/mL in an antifade solution (Appligene Oncor).

**Table 1**

<table>
<thead>
<tr>
<th>Case No./Sex/Age (y)</th>
<th>Diagnosis</th>
<th>Size (cm)</th>
<th>Site (Segment)</th>
<th>Hormone Use</th>
<th>CGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/22</td>
<td>FNH</td>
<td>5.6</td>
<td>S 7</td>
<td>None</td>
<td>No aberrations</td>
</tr>
<tr>
<td>2/F/40</td>
<td>FNH</td>
<td>2.0</td>
<td>S 7</td>
<td>None</td>
<td>No aberrations</td>
</tr>
<tr>
<td>3/F/43</td>
<td>FNH</td>
<td>9.0</td>
<td>Right lobe</td>
<td>None</td>
<td>No aberrations</td>
</tr>
<tr>
<td>4/F/47</td>
<td>FNH</td>
<td>3.5</td>
<td>S 7 and 8</td>
<td>None</td>
<td>No aberrations</td>
</tr>
<tr>
<td></td>
<td>HCC</td>
<td>16.0</td>
<td>S 5 and 6</td>
<td>None</td>
<td>+1q, –4p, +5, –7q22-q35, +12, –17p, +19q, –21q, –22q, possible –3 and –9</td>
</tr>
<tr>
<td>6/F/49</td>
<td>FNH</td>
<td>7.5</td>
<td>S 6</td>
<td>None</td>
<td>No aberrations</td>
</tr>
<tr>
<td>7/F/54</td>
<td>FNH</td>
<td>1.8</td>
<td>S 3</td>
<td>Climanest</td>
<td>No aberrations</td>
</tr>
</tbody>
</table>

FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; S, segment.
CGH Analysis

The CGH hybridizations were analyzed by using a fluorescence microscope (Axioplan 2, Zeiss, Jena, Germany) connected to a digital imaging system and evaluated with the ISIS-3 software program (MetaSystems, Altlussheim, Germany). Labeled tumor DNA (FNH; green) was hybridized with red-labeled corresponding DNA of normal liver tissue (cases 2, 4-6), and all tumor probes were hybridized together with red-labeled control DNA of a healthy donor on chromosomes of healthy male subjects.

Results

Histologically, all FNHs showed broad fibrous septa with centrally located vessels, proliferating ductules at the septal margins, and a variable amount of chronic inflammation. In each case, the liver cell plates of the FNHs were 1 to 2 cells thick. Cytologic or architectural dysplasias, including small cell or large cell change, were not found in the FNHs. The HCC in case 4 was a moderately differentiated, trabecular HCC, with strong cytoplasmic immunostaining for alpha fetoprotein (Image 1). No histopathologic change compatible with chronic diffuse liver disease, such as viral hepatitis, hemochromatosis, or alpha1-antitrypsin deficiency, was found in any of the 7 patients. In addition, the surrounding parenchyma showed no evidence of liver fibrosis or cirrhosis.

CGH of 7 FNHs identified aberrations in only 1 case (case 5) Image 2, including gains at chromosomes 1p33-p36.3, 9q34, 12q24.1-q24.3, 16, 17p, 17q25, 19, 20q, and 22 and losses at chromosomes 1p22-p31, 5q14-q23, 6q11.1-q23, 12q21, and 14q14-q31 and possible loss of 4q (Table 1). Aberrations also were found in the HCC of case 4, including gains at chromosomes 1q, 5, 12, and 19q and losses at chromosomes 4p, 7q22-q35, 9p, 17p, 21q, and 22q. Possible losses were found on chromosomes 3 and 9 (Table 1) Image 3. No genomic alterations were found in any of the nonneoplastic liver tissue specimens studied or in the FNH of case 4 (Table 1, Image 3).

Clinical and pathologic data for all 7 patients are summarized in Table 1. Two patients (29%) had a history of hormonal contraception use (Table 1).

Discussion

FNH is a hepatic lesion composed of hyperplastic hepatocytes in 2-cell-thick plates, subdivided into nodules by fibrous septa, which form stellate scars. As in our series, FNH is more common in women (82%-91% of FNHs) than in men. Of women with FNH, 38% to 94% have a history of oral contraceptive use, and, thus, it is not a prerequisite for the development of FNH. As in our series, the mean diameter of FNH is 5.0 to 5.9 cm (range, 2-15 cm); it occurs most commonly as a single lesion (approximately 80% of the cases), and it can affect any lobe. FNHs can be multicentric with 2 or more lesions. Multiple recurrent FNHs occurring in a single patient have been termed progressive FNH. In contrast with liver cell adenoma (LCA), FNH usually is asymptomatic; fewer than 20% of the patients show symptoms such as epigastric pain, discomfort, or a palpable mass. Spontaneous hemorrhage is rare. Results of liver function tests and serum levels of alpha fetoprotein usually are within normal limits. Occasionally, FNH may be associated with hepatic hemangioma, LCA, meningioma, astrocytoma, glioblastoma multiforme, telangiectasia of the brain, beryllium, and dysplastic systemic arteries. To the best of our knowledge, we describe the ninth case of synchronous FNH and HCC. In 5 cases, FNH was associated with fibrolamellar HCC. FNH may be considered an epiphenomenon due to an abnormal blood supply, possibly derived from the tumor vasculature, or a putative precursor lesion. Chen et al described an FNH associated with a classic HCC, and although both lesions were of clonal origin, as shown by human androgen receptor gene (HUMARA) analysis, the inactivated alleles in the FNH and in the HCC were different, and, thus, HCC probably did not derive from the FNH in that particular case. FNH commonly was associated with the fibrolamellar type of HCC, which might be because both lesions occur at a relatively young age (between 20 and 45 years), therefore, a causal relationship is not established.

In our case, the HCC did not grow within the FNH, and we did not find an intimate spatial relationship between FNH and HCC; thus, it is unlikely that an abnormal blood supply of the HCC resulted in the development of FNH and that the HCC developed within this particular FNH. The nontumorous liver showed no evidence of chronic diffuse liver disease, explaining the pathogenesis of HCC. In the West, approximately 75% to 90% of HCCs arise in cirrhotic livers, with chronic viral hepatitis, alcohol consumption, and hemochromatosis being major risk factors. HCCs occurring in noncirrhotic livers also have been attributed to chronic viral hepatitis, hemochromatosis, and alcohol consumption. However, we were unable to specify any of these as risk factors in our patient, and the pathogenesis of HCC remains obscure. In general, FNH is believed to be a hyperplastic response to an arterial malformation, and contradicting results have been reported about its clonal origin when the methylation pattern of the polymorphic X chromosome–linked androgen receptor gene (HUMARA) was analyzed: it was shown to be either monoclonal or polyclonal in origin. In contrast with FNH, LCAs and HCCs are always considered clonal expansions.
Image II Macroscopic and histomorphologic characteristics of synchronous focal nodular hyperplasia (FNH) and hepatocellular carcinoma (HCC). A, Cross-sections of FNH and HCC after fixation in formalin. B, The nonneoplastic liver shows a normal architecture (H&E, ×20). C, FNH with large vessels in a central fibrosis (H&E, ×4). Moderately differentiated, trabecular HCC (D, H&E, ×20) with expression of alpha fetoprotein (E, ×40).
Conflicting results about the clonality of FNH might be due to sampling errors. In contrast with LCA and HCC, FNH is, almost per definition, composed of different cell populations (hepatocytes, bile duct epithelium, and inflammatory infiltrates), and clonality might be related to any of these cell types, depending on the relative amount of cells in the sample used for analysis. Hypothetically, FNHs also may be both monoclinal and polyclonal in origin, but as yet no evidence supports this notion.

The synchronous occurrence of FNH and HCC in 1 patient, as in our series, raises the question of a common pathogenesis, in particular in a liver without evidence of chronic diffuse liver disease. HCC and FNH might respond to hormonal stimuli, but our patient gave no history of oral contraceptive use. We used CGH to study a putative underlying cytogenetic aberration that might help to explain the synchronous occurrence of FNH and HCC. While the HCC showed several chromosomal alterations, neither the nontumorous liver nor the FNH showed alterations. Thus, in this particular case, a common cytogenetic denominator was not found.

CGH has a sensitivity of 10 to 20 megabases (MB), and aberrations smaller than 10 MB escape detection. Thus, minor aberrations cannot be excluded. In contrast with FNH, LCAs have a small but well-documented risk to progress to HCC, and cytogenetic aberrations were found in a minority (20%) of LCAs. However, it is unknown whether these aberrations indicate a premalignant potential of LCAs, and further studies are necessary to clarify this issue. We found chromosomal alterations in only 1 FNH. Histopathologic examination revealed no evidence of dysplasia or small cell or large cell change in this case, and follow-up of this patient revealed no other malignant tumor, while the nontumorous liver was without evidence of a chronic liver disease. Because FNH is composed of different cell types, ie, hepatocytes, bile

Image 2: Histologic appearance of case 5, which showed genetic aberrations in focal nodular hyperplasia. The lesion showed a central stellate scar (A, azan, ×2) and is composed of different cell types (B, H&E, ×10; and C, H&E, ×40). Dysplastic changes were not found (C).
duct epithelium, and an inflammatory infiltrate, it is unclear which cell population contributed to this aberration, and the biologic significance is difficult to interpret. However, the diagnostic implications of this observation are straightforward. It has been suggested that CGH might help to differentiate between LCA and HCC. Based on our findings, CGH should be applied only to specimens in which FNH has been excluded definitively. CGH is of no use for separating LCA from FNH because both might show none or some cytogenetic aberrations.

Typical chromosomal aberrations have been described to occur in HCC, including gains on the long arm of chromosome 1 and loss of the short arm of chromosome 17. Both aberrations were found in our HCC. Additional genomic alterations, which cannot be called tumor type–specific, were detected and might be interpreted as nonspecific genomic instability of this particular tumor.

The synchronous occurrence of FNH and HCC in 1 patient probably is coincidental and in keeping with previous observations in which only 2 malignant transformations have been found in more than 800 FNHs on record. Thus, observation rather than resection can be considered adequate clinical management for FNH. However, cytogenetic aberrations might occur in FNHs, and CGH may be of limited diagnostic use for separating FNH from LCA or HCC.

Supported by institutional money from the Institute of Pathology, University of Magdeburg, and the Clinic for Gynecology, Christian-Albrechts University.

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References


