IHCA represents the largest subgroup of HCA and has been reported to be related with systemic disorders, such as obesity, metabolic syndrome, and alcohol abuse [7, 9]. One report mentioned that IHCA patients with a high body mass index (BMI ≥ 25) represent 60% of their study group in which the mean BMI is 28 [9]. Subgroups of HCA except the β-catenin gene-mutated type rarely show malignant transformation into hepatocellular carcinoma (HCC) although a recent study reported an increased risk in HCA occurring in overweight or obese male patients [10]. These findings suggest that in obese individuals the whole hepatic microenvironment is influenced by systemic factors that may favor tumor development, in accordance with the postulation that obesity increases the risk of cancer development [11].

In the present study, we investigated the histological features of the nontumorous liver parts of 32 resected IHCA specimens, to gain insight in the hepatic microenvironment in which IHCA develops, also because IHCA are often multifocal. Therefore, knowledge about the nonlesional liver tissue that corresponds to the remnant liver after tumor resection may influence the followup management of IHCA patients.

We found that although the lobular architecture is largely well preserved, the nontumorous liver frequently shares several abnormal features with the adenoma, such as sinusoidal dilatation and single arteries. Moreover, many cases also contain several foci of minute HCA-like areas with focal increase of CRP/SAA. These findings suggest that the nonlesional part of HCA-containing livers harbors changes that may potentially stimulate adenomatous growth. This is especially true for livers with multiple adenomas.

2. Patients and Methods

2.1. Patients. Thirty two patients, all of them females (mean age 33.5 ± 8.8 years), who underwent partial liver resection for IHCA, were included. Cases were selected on the availability of sufficient amount of adjacent nontumorous liver (AL) and/or distant nonlesional liver tissue (DL). The latter sample was taken at least 3 cm distant from the tumor.

2.2. Histology. A representative slide of the transformation area of tumor and adjacent nontumorous liver tissue (AL, $n = 32$) and one sample from the distant nonlesional part (DL, $n = 22$) were reviewed without knowledge of clinical data and the features of the corresponding tumor. Slides were stained with hematoxylin-eosin (HE) and Masson trichrome. The AL and DL samples were assessed separately for the following features: liver architecture, steatosis, steatohepatitis, sinusoidal dilation, single artery, and ductular reaction. Grading of steatosis and steatohepatitis was performed according to the scoring system for nonalcoholic steatohepatitis (NASH) proposed by Brunt et al. [12]. In summary, steatosis: 0 = absent; 1 = steatosis observed in up to 33%; 2 = more than 33% and less than 66%; 3 = more than or equal to 66% and steatohepatitis: 0 = absent; 1 = occasional ballooned hepatocytes, mild portal chronic inflammation; 2 = obvious ballooned hepatocytes, portal and intra-acinar chronic inflammation noted, mild to moderate; 3 = ballooning and disarray obvious with mild chronic inflammation, portal chronic inflammation, mild or moderate.

Grading of sinusoidal dilation followed the criteria mentioned by Rubbia-Brandt et al. [13]. Sinusoidal dilation: 0 = absent; 1 = centrilobular involvement limited to one-third of lobular surface; 2 = two-thirds lobular surface involved; 3 = complete lobular surface involved. Liver architecture is scored as preserved (1) or abnormal (0). Single artery and ductular reactions (DRs) are described as absent (0) or present (1). Single arteries are defined as arterial structures without accompanying bile duct and/or not localized in a portal tract structure. Assessment of DR is described below.

Table 1: Antibodies applied for immunohistology.

Antibody	Dilution	Retrieval methods	Company
β-catenin	1 : 100	Tris-EDTA	BD Transduction (USA)
GS	1 : 4000	Tris-EDTA	Millipore (USA)
CRP	1 : 200	Tris-EDTA	Abcam (UK)
SAA	1 : 200	Protease 8 min	Dako (DK)
CK19	1 : 100	Protease 12 min	BD Bioscience (USA)
CD34	1 : 20	Tris-EDTA	Dako (DK)
α-SMA	1 : 800	Tris-EDTA	Dako (DK)

2.3. Immunohistochemistry. The immunohistological expression of SAA/CRP on tumor tissue was already performed at an earlier, diagnostic stage to establish the diagnosis of IHCA according to the Bordeaux classification [7]. GS and β-catenin staining were also completed at the earlier diagnostic stage to assess possible β-catenin mutation. For the present study, AL and DL samples were stained according to the same protocol, and additional immunostaining with K19, CD34, and α-SMA was performed. K19 increased the feasibility to assess DR as the ductular structures were highlighted by K19 labeling. The presence of 4 or more ductular profiles per portal tract is regarded as the presence of DR [14].

CD34 visualized sinusoidal capillarization and single arteries, whereas α-SMA labeled myofibroblastic transformation of hepatic stellate cells. The antibodies used for the immunohistological staining are mentioned in Table 1 including the applied dilutions and retrieval methods.

3. Results

3.1. Architecture: Generally Well Preserved. In all AL and DL samples, the overall lobular architecture was largely well preserved. A normal distribution pattern of portal tracts and central veins was recognizable. The transition from lesional to nonlesional tissue was usually recognizable by the slightly pushing, irregular border of the nonencapsulated tumor, except in hemorrhagic or necrotic parts where a fibrous scar may have developed and form a capsule. The regular