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Correlations between ADC values and molecular markers of Ki-67 and HIF-1α in hepatocellular carcinoma

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ABSTRACT

Objective: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths. Cell proliferativity and hypoxia have important impact on the response to radiotherapy or chemotherapy. The purpose of this study was to investigate the association of apparent diffusion coefficient (ADC) values and the molecular markers Ki-67 and hypoxia inducible factor-α (HIF-α) in hepatocellular carcinoma (HCC).
Materials and methods: Forty-seven patients diagnosed with HCC were included in this study. All patients performed diffusion-weighted magnetic resonance imaging (DW-MRI) before any anticancer treatment. The ADC maps were automatically calculated on a Syngo workstation. The Ki-67 and HIF-1α expression were assessed by immunohistochemistry. The Pearson correlation test was used to assess the correlation between ADC values and Ki-67 and HIF-1α expression.
Results: Ki-67 staining was clearly identified based on the brown nuclear staining in tumor cells. High Ki-67 expression was correlated with low differentiation ($p = 0.028$). A significant correlation was observed between HIF-1α expression and maximum diameter ($p = 0.014$). The mean ADC value was $(0.983 \pm 0.21) \times 10^{-3}$ mm^2/s. The level of Ki-67 expression was correlated inversely with the ADC values ($r = -0.371$, $p = 0.01$). There was a significant positive correlation between the ADC values and HIF-1α expression ($r = 0.389$, $p = 0.007$).
Conclusion: The ADC values were observed to correlate significantly with the molecular markers Ki-67 and HIF-1α. Our results suggest that the ADC values on DW-MRI may be used as a measure of cell proliferativity and hypoxia in hepatocellular carcinoma.

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths [1]. Attempts have been made to predict the prognosis and response to therapy in patients with HCC. The nuclear Ki-67 protein is associated with cell proliferative activity, which may be indicative of tumor aggressiveness, and is expressed in all phases of the cell cycle except G0, with particularly high expression observed in the G2/M phase [2]. High expression of Ki-67 is associated with a higher tumor grade [3] and higher mortality [4]. Although HCC is one of the most hypervascularized tumor types with rich blood perfusion, the tumors still contain hypoxic regions, especially in patients with liver cirrhosis [5]. Hypoxia inducible factor-α (HIF-α) plays a crucial role in coordinating the response to hypoxic conditions [6,7]. High HIF-1α expression in HCC tissues is associated with capsular infiltration, portal vein invasion, and shorter survival of HCC patients [7,8].

Currently, we typically use pathological methods to obtain information about tumor hypoxia and proliferation. However, resection is currently limited to only limited patients who present with small nodules at diagnosis, lesions confined to the liver, well-preserved liver function and good performance status [9,10]. Most patients can be clinically diagnosed as HCC based on alpha-fetoprotein examination, contrast-enhanced computed tomography or magnetic resonance imaging (MRI). Tissue biopsy, an invasive unpleasant procedure, becomes unnecessary for those patients. Therefore, it will be useful to detect tumor hypoxia and proliferation uninvasively.

Diffusion-weighted MRI (DW-MRI) is a promising functional imaging tool for detecting and characterizing tumors [11,12]. In

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biologic tissue, the DWI signal is derived from the motion of water molecules [13]. The ADC values were calculated based on water protons experiencing different restrictions to diffusion inside cellular compartments [14]. Therefore, compartments with different cellular structures could be identified on ADC maps and may exhibit different ADC values [14,15]. Tumor proliferation and hypoxia leads to changes of cellular structures. We hypothesized that ADC values might correlate with tumor proliferation and hypoxia. Ki-67 and HIF-1α are the biomarkers for tumor proliferation and hypoxia. So the purpose of this study was to investigate the association between MRI features and the molecular markers of Ki-67 and HIF-1α.

2. Materials and methods

2.1. Patients

This study was approved by the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University, and informed consents were obtained from all patients. We searched our radiologic database for liver MRI examinations performed between October 2009 and July 2014. The patients were included according to the following criterias: first, the HCC diagnosis was confirmed by pathology; second, MRI examnation were performed before any anticancer treatment, such as surgery, trans-arterial chemoembolization (TACE), or targeted therapy; third, after MRI examinations, surgery or biopsy was performed within 1 week; and forth, performing the alpha fetoprotein (AFP) examination before treatment.

2.2. MRI protocols

All patients were imaged in the supine head-first position on a 3.0T system with a surface phased-array coil (Magnetom Verio, SIEMENS, Germany). Axial and coronal T2-weighted (T2W) turbo spin-echo imaging (echo time[TE], 96–104 ms; repetition time[TR], 3000–4000 ms; section thickness, 3–4 mm; echo train length, 16; field of view, 38 cm; matrix, 320 × 256) was performed. Transverse respiratory triggering DW imaging was performed using the single-shot echo-planar imaging technique (b values, 0 and 800 s/mm^2; TR, 4000; TE, 73 ms; matrix, 160 × 120; Bandwidth 2442 Hz/Px; Pixel size, 3.7 × 3.0 mm; NEX, 3); the center of the slice groups, field of view and section thickness were the same as those used for the axial T2W images.

2.3. MR image analysis

The ADC maps were automatically computed on a Syngo workstation (Syngo Multimodality Workplace, Siemens, Germany). Two abdominal radiologists with 15 and 12 years of experience, who were blinded to the pathological diagnosis drew the regions of interest (ROIs) by consensus. Each ROI was manually contoured (free-hand ROIs) on ADC map, excluding haemorrhage, necrotic or cystic portion, which were identified on the T2-weighted images (T2WI) and the contrast-enhanced T1-weighted (T1WI). When the lesion was not visualized well on the DW images, the T2WI, T1WI and the contrast-enhanced T1WI were reviewed for the accurate placement of the ROI on the lesion by visual correlation of the image sets. A lesion contained more than one ROI if the tumor was large. The mean ADC value of a lesion was calculated by averaging the ADC values of all the voxels in all ROIs of a lesion (Eq. (1)), where n was the number of ROIs, ADC_i was the mean ADC of the ith ROI of a lesion, and S_i was the area of the ith ROI of a lesion.

$$\text{Mean ADC} = \frac{\left(\sum_{i=1}^{n} ADC_i S_i\right)}{\sum_{i=1}^{n} S_i} \quad (1)$$

2.4. Immunohistochemical analysis of Ki-67 and HIF-1α

Ki-67 and HIF-1α were assessed using paraffin-embedded tissue samples which were cut into 5-μm-thick slices. Briefly, all sections were deparaffinized and antigen was retrieved under high pressure for 120 s. Nonspecific binding was blocked by serum at 37 °C for 15 min (Beijing Zhongshan Golden Bridge Biotechnology Company, China). The sections were stained with primary monoclonal rabbit anti-human HIF-1α antibody (Abcam, Cambridge, UK) or monoclonal mouse anti-human Ki-67 antibody (Beijing Zhongshan Golden Bridge Biotechnology Company, China) in a humidified chamber at 37 °C for 60 min. Then the specimens were incubated with secondary goat anti-rabbit antibody or goat anti-mouse antibody, respectively (Beijing Zhongshan Golden Bridge Biotechnology Company, China) at 37 °C for 30 min. Ki-67 and HIF-1α expression were visualized using 3, 3′-diaminobenzidine (DAB) followed by counterstaining with hematoxylin.

Two independent observers who were insensible of the clinicopathological parameters performed the scoring according to a previously validated scoring system. Ki-67 was considered positive when the cell nuclei were stained brown–yellow and was determined semiquantitatively as low (10% or less immunopositivity) or high (>10% immunoreactive cells) [16]. HIF-1α was classified as positive when the cell cytoplasms were stained brown–yellow. HIF-1α expression was determined by assessing the percentage of tumor cells with cytoplasmic staining and by monitoring the staining intensity using the following classification system: low, no staining and staining in <10% of tumor cells with weak stain-

Table 1
Patient characteristics.

Characteristics	Values
Median age	53
Sex	
Male	35
Female	12
Child-Pugh classification	
Grade A	40
Grade B	5
Grade C	2
Differentiation	
Low	10
Moderate	25
High	12
AFP	
<20 ng/ml	18
≥20 ng/ml	29
Tumor location	
Left	16
Right	31
Maximum diameter	
<3 cm	20
≥3 cm	27
Tissue type	
Biopsy tissues	17
Resected regimens	30