High mobility group A1 is expressed in metastatic adenocarcinoma to the liver and intrahepatic cholangiocarcinoma, but not in hepatocellular carcinoma: its potential use in the diagnosis of liver neoplasms

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Introduction

The high mobility group A (HMGA) family of proteins in mammals is known to consist of four proteins: HMGA1a, HMGA1b, HMGA1c, and HMGA2. The first three of these proteins are generated from a single functional gene, i.e., HMGA1 (formerly HMGI(Y)), while the last one is a product of a separate gene, i.e., HMGA2 (formerly HMGI-C).1,2 The HMGA proteins are nonhistone nuclear proteins, which bind to AT-rich regions in the minor groove of DNA via three AT-hook domains, and are thought to affect the transcription process by acting as architectural proteins.3,4 Recent studies demonstrated an important role for the HMGA1 proteins (formerly HMGI(Y) proteins) in regulating gene expression,5–10 although they have no transcriptional activity perse.11 The HMGA1 proteins, however, participate in the assembly of protein complexes on the promoters of several inducible genes, and have thus been defined as architectural transcriptional factors.5,9,12–14

HMGA gene expression is negligible in normal adult tissues, being essentially restricted to embryonic development.15,16 The HMGA1 gene/protein expression level, however, has been demonstrated to be elevated in many human neoplasms originating from a variety of tissues, including the thyroid, prostate, uterus, colorectum, and pancreas.17–26 These findings indicate the critical role(s) of the HMGA1 proteins not only in normal cell proliferation and/or differentiation but also in tumorigenesis and/or tumor growth. Moreover, HMGA1 has been reported to be an important c-Myc target gene involved in
neoplastic transformation. Further investigations have actually shown that high levels of HMGA1 mRNA expression are directly correlated with metastatic progression in several tumor cell lines. We have also demonstrated a significant correlation between the levels of HMGA1 protein expression and factors closely associated with a poor prognosis in patients with colorectal cancer. These data support the idea that HMGA1 proteins could be a potential target molecule for gene treatment, as well as a diagnostic marker for a wide variety of malignancies.

We previously demonstrated that colorectal carcinoma and pancreatic carcinoma express high levels of HMGA1. However, HMGA1 expression has not yet been studied in hepatic tumors. In the present study, we analyzed HMGA1 expression in hepatic primary and metastatic tumors in order to investigate whether determination of the HMGA1 expression level could provide any diagnostic advantages in the pathological diagnosis of hepatic tumors. To this end, HMGA1 expression was determined at the protein level in hepatocellular carcinoma, intrahepatic cholangiocarcinoma, and metastatic adenocarcinoma to the liver, by immunohistochemical analysis.

Materials and methods

Tissue samples

Tissue samples were obtained at the time of surgery at the First Department of Surgery, Kyrin University Hospital, between January 1990 and March 2001. Written consent regarding inclusion of the removed tissues in the study was obtained from the patients. The tissues were fixed in 10% neutral buffered formalin within 4 h after surgical removal, sectioned into blocks, and embedded in paraffin. The tissue samples obtained included 21 samples of metastatic adenocarcinoma to the liver (15 metastatic tumors from colorectal carcinoma and 6 metastatic tumors from pancreatic carcinoma), 5 samples of intrahepatic cholangiocarcinoma, and 20 samples of hepatocellular carcinoma (HCC), together with 32 samples of adjacent normal hepatic tissues.

Immunohistochemical analysis

Immunohistochemical examinations were performed by the avidin-biotin complex immunoperoxidase technique, using an Avidin-Biotinylated Enzyme Complex kit (Vector Laboratories, CA, USA). A primary rabbit polyclonal antibody against an HMGA1-specific synthetic peptide, corresponding to the NH2-terminal region of the molecule (Santa Cruz Biotechnology, CA, USA) was used in this study. In brief, paraffin sections (4 m) were cut, transferred onto Matsunami Adhesive Silan (MAS)-coated slides, deparaffinized in xylene, and rehydrated through graded alcohol series. The sections were subjected to microwave antigen retrieval in citrate buffer in a calibrated microwave at high power seven times, each time for 3 min, followed by quenching of the endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol. After a rinsing with phosphate-buffered saline (PBS), the sections were incubated with normal goat serum for 20 min at room temperature to block nonspecific binding, and then incubated with the primary anti-HMGA1 antibody at a dilution of 1:100 for 14 h at 4°C. After being washed in PBS with 0.2% Triton X-100, sections were further incubated with biotinylated anti-rabbit IgG for 30 min at room temperature, followed by washes in PBS with 0.2% Triton X-100. Subsequently, sections were incubated with streptavidin-biotin-conjugated peroxidase for 30 min at room temperature and washed in PBS with 0.2% Triton X-100, followed by visualization of the HMGA1 proteins by incubation of the sections with 3,3'-diaminobenzidine. The slides were then counterstained with Mayer’s hematoxylin, dehydrated in a graded alcohol series, cleared in xylene, and mounted. As the positive control, a pancreatic carcinoma tissue specimen overexpressing HMGA1 proteins was processed in a similar manner. Negative control staining was carried out by replacing the primary antibody with normal rabbit serum under the same experimental conditions. The immunostained slides were evaluated microscopically by a single investigator (N.A.) according to the criteria previously published, without prior knowledge of the clinical data for each case. The percentage of HMGA1-positive cells was scored by counting approximately 300–1000 tumor cells. Immunohistochemical evaluation was considered positive when HMGA1 nuclear immunoreactivity was detected in more than 20% of the cells according to the criteria previously published.

Results

HMGA1 expression in metastatic lesions from colorectal and pancreatic carcinomas was analyzed first because these carcinoma species have been shown to express high levels of HMGA1 gene/protein at the primary sites. Among the 15 samples of metastatic lesions from colorectal carcinoma, 8 showed positive HMGA1 immunoreactivity (53.3%). In these HMGA1-positive samples, the HMGA1 nuclear immunoreactivity tended to be distributed unevenly within the tumor lesion (Fig. 1a). In contrast, all the metastatic lesions from pancreatic carcinoma showed positive HMGA1 immunoreactivity characterized by intense nuclear