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Increased Expression of Histone Deacetylase 2 is Found in Human Hepatocellular Carcinoma

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Abstract

Accumulated evidences have established that aberrant regulation of histone deacetylases (HDACs) is one of major causes for development of human malignancies. Mammalian HDACs can be divided into three subclasses consisting of 11 homologous of HDACs and 7 of sirtuins, but little is known about HDAC2 causes for carcinogenesis in solid tumors. Here, in order to investigate the roles of HDAC2 in carcinogenesis of liver cancer progression, we analyzed the expression of HDAC2 in 62 human hepatocellular carcinomas by utilizing Immunohistochemistry. Moderate to strong expression of HDAC2 was found in 54 (87%) out of total 62 tumors. The majority of positive tumors were detected in nucleous, but normal hepatocytes did not express of HDAC2 or showed weak positive staining. Interestingly, we were also noted that HDAC2 expression was appeared to be associated with aggressiveness of the tumors by the fact that HDAC2 expression was observed with significances in high grade tumors (Edmonson grade II, III). Taken together, we found the aberrant expression of HDAC2 in hepatocellular carcinomas, and this suggests that HDAC2 may play an important role in the development of liver cancer.

Keywords: Hepatocelluar carcinoma, Histone deacetylase 2, Edmonson grade

Hepatocellular carcinoma (HCC) is one of most common malignancies in the world[1,2]. Recent studies have found that genetic alterations of tumor suppressor genes or oncogenes such as p53, β-catenin, and AXIN1 were involved in hepatocarcinogenesis[3,4], but the frequency of mutation of these genes is appears to be very low in HCC patients. Furthermore, it is unlikely clear how these genetic changes precisely reflect the clinical characteristic of individual patients in HCC patients. Therefore, the molecular events underlying HCC in most patients still remains unknown. Accumulated evidence have established that carcinogenesis is a multi-step process that is associated with alterations in cellular oncogenes and tumor suppressor genes necessary for malignant transformation[1,5,6]. In liver carcinogenesis, it has also suggested that multiple genetic alterations are responsible for the development and progression of gastric cancer, and alterations in specific genes that play important roles in diverse cellular functions such as cell adhesion, signal transduction, differentiation, development or DNA-repair have been identified[7]. However, many of these putative oncogenes or tumor suppressors are still remained to be elucidated for future targets of therapeutic intervention in hepatocelluar carcinoma.

The recruitment of histone acetyltransferases (HAT) and HDACs is considered as a key element in the dynamic regulation of many genes playing important roles in cellular proliferation. Recently, histone deacetylase (HDAC) inhibitors are emerging as an exciting new class of potential anticancer agents for the treatment of solid and hematological malignancies and a number of HDAC inhibitors have been identified that induce tumor cells to undergo growth arrest, differentiation, or apototic death[8,9]. In fact, several syndromes associated with increases risk for cancer have mutation in a HAT or aberrant recruitment of HDACs including Rubinstein Taybi syndrome, therapy related acute myelogenous leukemias and lymphomas, myelodysplasia and some types of colorectal and gastric carcinoma. The HDACs comprises class I and II emzymes of the deaceylase superfamily, and the class III enzymes (sirtuins) are HDACs in yeast[10,11]. Currently, only limited information is available about the isoenzyme-specific or redundant biological functions of these HDACs. Histone deacetylase 2 (HDAC2) has been identified with ~50% identitical in DNA and protein sequence compared with HDAC1 or HDAC3, and thus belongs to class I HDACs. Like HDAC1 and HDAC3, HDAC2 was also suggested that it may participate in a large complex that mediat-

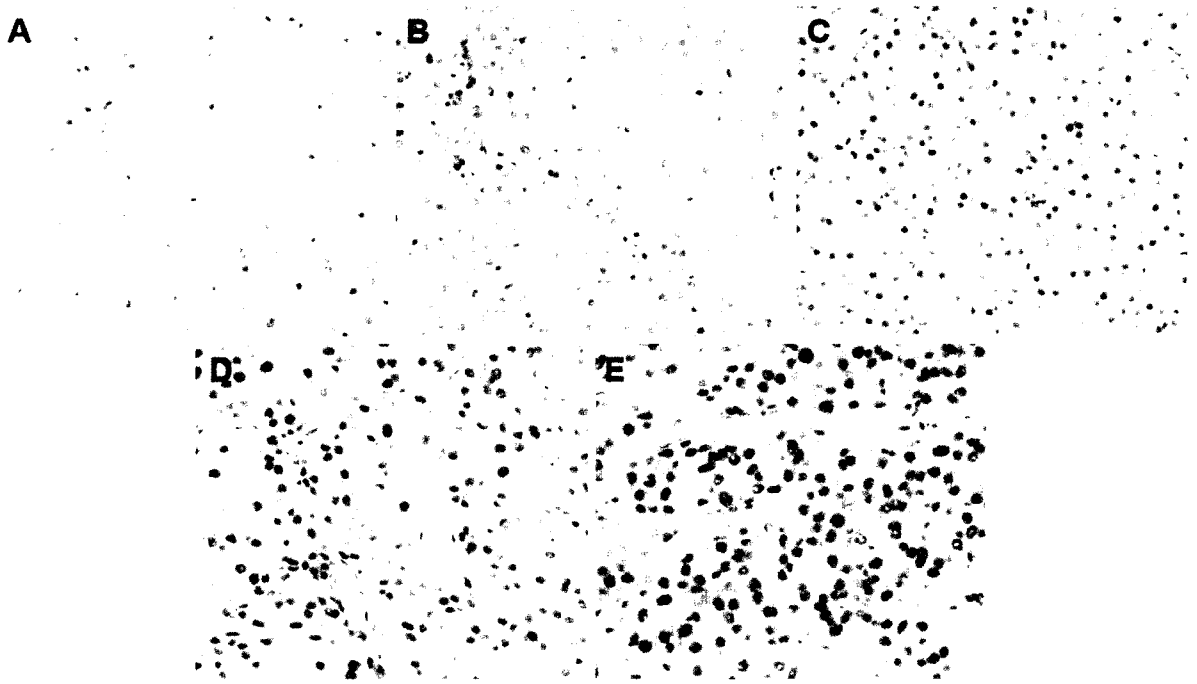

Fig. 1. Expression of HDAC2 in hepatocelluar carcinoma by immunohistochemistry. A, Hepatocytes, negative reaction was seen in normal liver hepatocytes. B, Hepatocytes with cirrhotic background, very weak positive for HDAC2 antibody staining. C. Edmonson grade 1-3 of HCC showed moderate to strong immunopositivity in the nucleous of the tumor cell. (Original magnification: ×200)

es a wide variety of repression system in human, and revealed that HDAC2 was ubiquitously expressed in many different tissues 12. Indeed, recent study has suggested that increased HDAC2 expression was found and was enhanced by loss of APC in the human colorectal cancer. This was the first evidence that HDAC2 could be the relevant potential target in cancer therapy. However no approaches have made to other type of solid tumor, thus we evaluated expression pattern of HDAC2 in surgically resented hepatocellular carcinomas by using immunohistochemisty to address HDAC2 as a potential therapeutic target in liver cancer.

Immunohistochemistry for the HDAC2

In immunohistochemistry, normal hepatocytes showed negative for HDAC2 antibody staining, but very weak expression of HDAC protein in hepatocytes with cirrhotic background of liver (Fig. 1A and B). However, continuous increasing of HDAC2 expression was observed overt cancer. As shown in Fig. 1C and D, hepatocellular carcinomas from Edmonon grade I-III (HCC G1-3) showed positive for the HDAC2 expression. Interestingly, over expression of HDAC2 was detected in 5 (83%) of 6 HCC G1, 30 (85%) of 35 HCC G2 and 19 (90%) of 21 HCC G3, respectively (Table 1). Overall, most of, except with a few cases, HCCs showed moderate to strong positive for the HDAC2. Furthermore, positive responsiveness was increasing form HCC G1 to HCC G3 (Table 1). It seems that HDAC2 protein expression is closely related to the progression of liver cancer.

Table 1. Summary of HDAC2 expression in human hepatocellular carcinomas.

Histologic grade	HDAC2 expression*			
	–		+	++
G1	+++ 1/6(16.7)	3/6(50)	2/6(33.3)	0/6(0)
G2(%)	5/35(143)	6/35(17.1)	21/35(60)	3/35(8.6)
G3(%)	2/21(9.5)	0/21(0)	4/21(19)	15/21(31.5)

*Intensity of expression is described as – (negative), + (weak positive), + (moderate positive) and +++ (strong positive).

Discussion

In the present study, we have investigated that HDAC2 was frequently expressed in hepatocellular carcinomas in contrast with normal hepatocytes nega-

tive or very weak positive (Fig. 1 and Table 1). Increasing expression of HDAC2 on liver cancer grade also suggested that expression pattern of HDAC2 may lie in the aggressiveness of liver cancer. Although further investigations regarding the capability of HDAC2 on tumorigenic potential of liver cancer should be provided, this finding would be useful clue for providing a new therapeutic target in liver cancer treatment.

Cancer cells may have aberrant gene expression by multiple causes such as genetic alterations (mutation, homozygous deletion, loss of heterozygosity, etc), cytogenetic changes (monosomies, trisomies, double minutes, etc) and epigenetic changes[13]. Epigenetic gene silencing takes place by two different mechanisms by which hypermethylation of CpG islands in promoter region of genes or promoter hypoacetylation of histones caused by recruitment of HDACs on the absence of DNA methylation[14,15]. Thus HDACs play an important role in regulating tumor suppressors and the aberrant recruitment of HDACs leads to the transcription repression of these genes. Recent investigation has revealed that HDCA2 expression is augmented by transcriptional activation of c-Myc through the β-catenin accumulation as consequence of loss of function mutation of the tumor suppressor *adenomatosis polyposis* coil (APC) in colorectal tumor[16]. This was the first report showing the transcriptional activation of HDAC2 could be regulated by the activation of Wnt/ β-catenin signaling pathway in a solid tumor carcinogenesis mechanism. Furthermore, they also showed that adenoma formation was greatly reduced by the treatment of Valproic acid, a potent selective HDAC2 inhibitor using *in vivo* mouse model system. In gastric caner, many of reports have also suggested that Wnt pathway activation contributed to the carcinogenesis of gastric tumor, and this implies the possibility of regulation of HDAC2 expression by Wnt pathway in gastric cancer. In addition, among the HDAC family, as only HDAC2 has a specific inhibitor such as valproic acid, it can be easily assessed as a therapeutic target for gastric cancer too. However the frequency of over-expression of HDAC2 was observed by around 65% in tumor, but the nuclear accumulation of β-catenin has been seen by around 30% frequency reflecting that there might be another activation mechanism of HDAC2 which by-pass the β-catenin stabilization[17]. Therefore the molecular mechanisms by which activate HDAC2 expression in gastric cancer remains to be elucidated.

Patients with HCC have a poor prognosis because most HCCs are detected at a stage too late for curative treatment. Therefore early detection of small HCC or precancerous lesions appears to be a challenge. And unfortunately, morphologic and molecular features of premalignant hepatic lesions are far from being fully elucidated and uniformly accepted. In our study, HDAC2 expression was observed in almost all HCCs with a few exceptions. This suggests that up-regulation of HDAC2 may be required for the acquisition of neoplastic property for the transformation of normal hepatocytes or it may be essential to be immortalization by normal cells.

In conclusion, the results presenting in this study define the expression pattern of HDAC2 in HCCs. Differential expression of HDAC2 in some malignant HCCs and its correlation to aggressiveness of cancers suggested that aberrant expression of HDAC2 might contribute to development of liver cancer providing a new therapeutic intervention for liver cancer.

Methods

Tissue Samples

Formalin-fixed and paraffin-embedded samples were obtained from surgical resections of 62 hepatocellular carcinomas. Thirty-five were of Edmoson grade II and twenty-one were of Edmonson grade III. Two pathologists screened histological sections and selected areas of the representative tumor cells. Three tissue cores (0.6 mm in diameter) were taken from each tumor samples and placed in a new recipient paraffin block using a commercially available microarray instrument (Beecher Instruments, Micro-Array Technologies, Silver Spring, MD), according to established methods[18]. One cylinder of normal hepatocyte with cirrhotic background adjacent to each tumor was also transferred to the recipient block.

Immunohistochemistry for the HDAC2

For immunohistochemical analysis, 2 μm sections were cut the day before use and stained according to standard protocols. To maximize the signal on immunohistochemistry, two strategies were used in the present study, i.e. antigen retrieval in citrate buffer, and signal amplification with biotinylated tyramide. For the former, heat-induced epitope retrieval was conducted by immersing the slides in Coplin jars filled with 10 mmol/L citrate buffer (pH 6.0) and boiling the buffer for 30 min in a pressure cooker (Nordic Ware, Minneapolis, MN) inside a microwave oven at 700 W; the jars were then cooled foe 20 min. For the latter, the Renaissance TSA indirect kit (NEN Life Science, Boston, MA), which included streptavidin-peroxidase and biotinylated tyramide, was used. After rinsing with PBS, the slides were treated

with 1% H_2O_2 in PBS for 15 min at room temperature to abolish endogenous peroxidase activity. After washing with TNT buffer (0.1 mol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl and 0.05% Tween 20) for 20 min, the slides were treated with TNB buffer (0.1 mol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl and 0.5% blocking reagent)[18,19]. Sections were incubated overnight at 4°C with the antibody (1/500 dilution) for HDAC2 (Upstate cell signaling solutions, Lake Placid, NY), and detection was carried out using biotinylated goat anti-rabbit antibody (Sigma, St. Louis, MO), followed by incubation with peroxidase-linked avidin-biotin complex. Diaminobenzidine was used as chromogen, and slide was counterstained with Mayer's hematoxylin. Because normal gastric mucosa showed focal positivity in less than 20% of cells, tumors were interpreted as positive when at least moderate to strong cytoplasmic staining was seen in over 20% of the cancer cells. The immunoreactivity was evaluated initially in three groups: high positive (++), >50%; low positive (+), 20-30%; and negative, <20% of the cancer cells. The results were reviewed independently by 2 pathologists. As negative controls, the slide was treated by replacement of primary antibody with non-immune serum.

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