

Changes of integrin expression in rat hepatocarcinogenesis induced by 3'-Me-DAB

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Abstract

AIM To investigate the expression of integrins in rats liver during 3'-Me-DAB induced hepatocarcinogenesis and to find out the relationship between integrins and liver cancer metastasis.

METHODS The expressions of integrins α_1 , α_2 , α_3 and α_5 and epidermal keratin (EK) were observed by immunohistochemical PAP method.

RESULTS In the normal liver tissues, hepatocytes express integrins α_1 and α_5 and in the bile duct epithelium, EK. In liver cirrhosis, hepatocytes highly express integrins α_1 , α_2 , α_3 and α_5 and in hyperplastic bile duct epithelium, integrins α_1 , α_5 and EK. Expression of integrins α_1 , α_2 , α_3 and α_5 were obviously decreased in the preneoplastic nodules and primary carcinoma but expressions of integrins α_1 and α_5 in metastasis in the lung and diaphragm were higher than those in primary carcinoma.

CONCLUSION Integrins α_1 and α_5 may play a major role in chemically induced hepatocarcinogenesis and metastasis in rats.

INTRODUCTION

The integrin superfamily of heterodimeric cell-surface receptors composed of distinct α and β subunits mediates the adhesion of cells to the extracellular matrix and in some instance, the intercellular adhesion. The integrins are thought to play important roles in differentiation and development, cell migration, and the complex process of tumor cell invasion and metastasis, as well as adhesion^[1]. Alterations in integrin expressions upon malignant transformation and in naturally occurring human malignancies are now well established. However, the mechanisms that give rise to altered integrin protein expression following malignant transformation are poorly understood. We investigate the expression of integrins α_1 , α_2 , α_3 and α_5 in rat liver during 3'-Me-DAB induced hepatocarcinogenesis and expect to find out the relationship between integrins and liver cancer metastasis.

MATERIALS AND METHODS

Animal model^[3]

Male Wistar rats ($n=100$, provided by Experimental Animal Center, Shanghai Medical University), weighing 100g-150g, were divided into four groups: group A ($n=44$), fed with low choline maize powder and 0.03% 3'-Me-DAB for 12 weeks; group B ($n=28$), fed with low choline maize powder only for 12 weeks and group C ($n=28$), fed with standard diet only. After 12 weeks, the rats of group A and B were fed with standard diet. Four to rats were killed in each group at week 3, 6, 9, 12 and 16 and 8-12 at week 20.

Tissue preparation

Small pieces of liver samples (including tumor and metastasis) were divided in two parts, one embedded in O.C.T (Miles, USA), rapidly frozen and stored at -70°C , and the other was fixed with 10% neutral buffered formalin, embedded in paraffin and used for routine histological examination.

Immunohistochemistry

The antibodies against integrins α_1 , α_2 , α_3 and α_5 were purchased from Chemicon International INC and those against epidermal keratin (EK) from Nichi Lei Co. Tokyo, Japan. The rabbit PAP kits were prepared by our department^[4]. Integrins α_1 , α_2 , α_3 and α_5 and EK in normal liver, liver cirrhosis

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and liver tumor were detected by PAP method. Briefly, 5 μ m cryostat sections of the frozen tissues were fixed with cold acetone and 10% neutral buffered formalin (9:1) for 10min, and washed in 0.01mol/L phosphate- buffered saline (PBS, pH 7.4) for 5 times, each for 3min. The sections were treated with methanol (containing 0.2 mL/L H₂O₂) at 37°C for 30min and washed in PBS, and then incubated in PBS with 100mL/L normal goat serum at 37°C for 30min. The sections were incubated with rabbit anti-integrins α_1 (1:1200 dilution), α_2 (1:1200 dilution), α_3 (1:1200 dilution), α_5 (1:1500 dilution) and EK (1:1000 dilution) respectively at 37°C for 30min and the n at 4°C overnight. The sections were washed in PBS and incubated with goat anti-rabbit IgG (1:200 dilution) at 37°C for 40min. After washing in PBS, the sections were incubated with rabbit PAP complex (1:200 dilution). The color was developed with 0.5g/L 3, 3'-diaminobenzidine/0.5mL/L H₂O₂/0.05 mol/L PBS (pH 7.6) for 10min. Normal rabbit serum instead of the specific primary antibodies was used as negative control.

Semiquantitation of the results

Only cells with membranous staining were considered; cells with intense dotlike cytoplasm immunoreactivity caused by endogenous peroxidase were ignored. The intensity of staining was divided into +++ (strong), ++(moderate), + (weak), +/- (equivocal), - (negative). Variable patterns were indicated by combining the two extremes of staining intensity, e.g. +++/+/-.

RESULTS

Morphologic changes

Liver cancer model of the rats in group A was successfully induced by fed 3'-Me-DAB with low choline maize powder. In the early experiment (week 1-9), the main morphologic changes were steatosis and necrosis of hepatocytes, proliferation of oval cells and cholangiofibrosis. In the middle of the experiment (week 10-16), massively proliferated oval cells, and extensive areas of cholangiofibrosis were visible. In some livers, few hepatocytes remained; they were either entrapped in cholangiofibrotic structures or located at the peripheries of these lesions. Then, liver cirrhosis and preneoplastic nodules were obvious. At the end of the experiment, most of the rats in group A had liver tumor, mainly mixed hepatocarcinoma (HCC) and cholangiocarcinoma (CCC). Tumor metastasized to the lung, spleen and diaphragm in some rats (Figure 1). There were no obvious morphologic changes in the livers of rats in group B and C.

Expression of integrins and EK

The overall staining patterns are shown in Table 1.

Table 1 Pattern of integrins and EK in normal liver, liver cirrhosis and liver tumor

	α_1	α_2	α_3	α_5	EK
Normal liver tissue					
Hepatocytes	+	-	-	+	-
Sinusoidal endothelial cells	+	-	-	+	-
Bile duct epithelium	-	-	-	-	+++
Liver cirrhosis					
Hepatocytes	+++	++	++	+++	-
Sinusoidal endothelial cells	++	+	+	++	-
Oval cell	+/-	+/-	+/-	+/-	+++/-
Hyperplastic bile duct epithelium	++	-	-	++	+++
Preneoplastic nodules	+	+/-	+/-	+	-
Tumor					
Primary	-/+	-	-	-/+	+++/-
Metastasis	+++/-	-	-	+++/-	+++/-

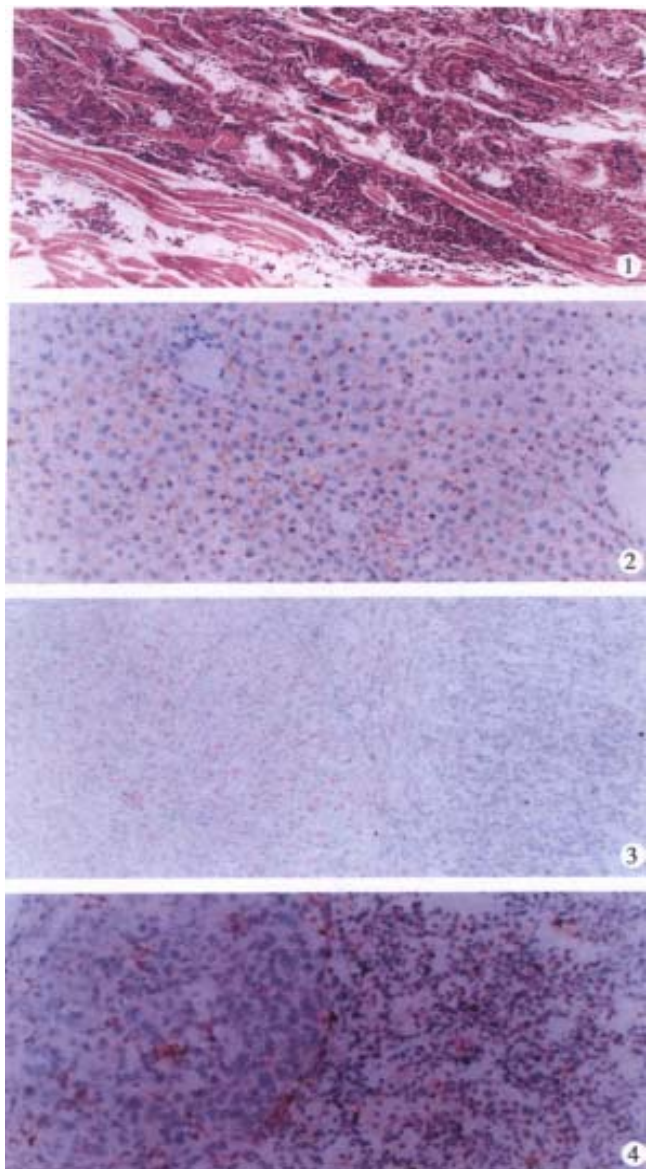


Figure 1 Tumor metastasis in diaphragm. HE×200

Figure 2 In liver cirrhosis, the expression of integrin α_1 was increased in hepatocytes and sinusoidal endothelial cells. PAP×200

Figure 3 Expression of α_1 was obviously decreased in the primary carcinoma(right) and in para-tumor liver cirrhosis(left), the expression of integrin α_1 was increased in hepatocytes and sinusoidal endothelial cells. PAP×100

Figure 4 Expression of α_1 in metastasis in the lung was higher than in primary carcinoma. PAP×200

In the normal liver tissues, integrins α_1 and α_5 were weakly expressed in hepatocytes and sinusoidal endothelial cells but not in bile duct epithelium. The predominant staining was located on the sinusoidal side plasma membranes of hepatocytes. EK was highly expressed in the bile duct epithelium but not in hepatocytes.

In liver cirrhosis, the expression of integrins α_1 , α_2 , α_3 and α_5 were increased in hepatocytes and sinusoidal endothelial cells but the distribution did not significantly change (Figure 2). However, hyperplastic bile duct epithelium only moderately expressed α_1 and α_5 . Oval cells were equivocal on expression of integrins but most of them highly expressed EK.

Expression of integrins α_1 , α_2 , α_3 and α_5 were obviously decreased in the preneoplastic nodules and primary carcinoma (Figure 3) but expression of integrins α_1 and α_5 in metastasis in the lung and diaphragm were higher than in primary carcinoma (Figure 4).

DISCUSSION

The β_1 subgroup of the integrin superfamily of cell-surface receptors (also known as VLA integrins) contains six heterodimers (VLA-1 to VLA-6), in which different α -subunits share a common β_1 subunit. They serve as receptor for extracellular matrix components as laminin (VLA-1, VLA-2, VLA-3, and VLA-6), collagen (VLA-1, VLA-2, and VLA-3), and fibronectin (VLA-3, VLA-4, and VLA-5) and are involved as counter-receptors for other cell-surface molecules (VLA-4 recognizes the vascular cell adhesion molecule-1 [VCAM-1]) in a cell-to-cell type of interaction^[2].

The integrins are thought to play important roles in differentiation and development, cell migration, and the complex process of tumor cell invasion and metastasis, as well as adhesion. The integrin β_1 and fibronectin were mainly distributed along sinusoids in normal liver, and the expression was weak in adult normal liver^[5]. The distribution did not significantly change during the regeneration and proliferation, but the expression was increased. They acquire some characteristics of fetal and neonatal hepatocytes, including alternations in cell surface properties, such as the composition of glycoconjugates, and the level of receptors for hormones and growth factors and of proteins involved in cell-cell contacts. In the early phase of liver carcinogenesis, proliferation of oval cells, cholangiofibrosis, proliferation of hepatocytes and the extracellular matrix (cirrhosis) were obvious. The high expression of integrins plays an important role in cirrhosis^[6].

Alterations in integrins receptor expression upon malignant transformation and naturally occurring human malignances are now well established^[7,8]. It was one of the properties of tumor cells that the expressions of integrins and fibronectin were decreased^[9]. Integrins such as fibronectin receptor transduce important growth inhibitory stimuli from the extracellular matrix. Integrins are involved not only in the adhesive, motile and invasive behavior of tumor cells but also in their growth regulation.

Expression of integrins α_1 , α_2 , α_3 and α_5 was obviously decreased in the preneoplastic nodules and primary carcinoma but expression of integrins α_1 and α_5 in metastasis in the lung and diaphragm was higher than in primary carcinoma. Integrins α_1 and α_5 may play a major role in chemically induced hepatocarcinogenesis and metastasis in rats, and it was necessary for tumor cells to adhere to the extracellular matrix in metastatic focus^[10,11].

EK is a mark of epidermal cells. EK was highly expressed by the bile duct epithelium but not by hepatocytes and might be a helpful tool in the differential diagnosis between HCC and CCC. Some oval cells expressed EK but some not. It suggested that oval cells are the progeny of hepatic stem cells, which might differentiate into preneoplastic parenchyma cells and might give rise to HCC.

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