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Expression of metastasis suppressor gene *nm23* in human hepatocellular carcinoma

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Abstract

AIM: To investigate the relationship between the expression of *nm23-Hi* mRNA and the metastatic potential of hepatocellular carcinoma (HCC).

METHODS: The expression of *nm23-H1* mRNA was detected in 24 cases of HCC by *in situ* hybridization using digoxigenin-labeled nm23-H1 antisense cRNA probe. Twenty-four HCC specimens were divided into two groups according to the following criteria: (1) metastasis in portal lymph nodes; (2) the number of tumors in the liver; (3) cancerous emboli in the portal vein; and (4) the existence of satellite lesions. We named those meeting criteria (1) or (2) and (3), or (3) and (4) high metastatic potential (n = 6); and the others formed the low metastatic potential group (n = 18).

RESULTS: Positive results of *in situ* hybridization showed granules or masses in the cytoplasm. In the low metastatic potential group strong staining was obtained in ten specimens, while in the high metastatic potential group there was none. Three negative results were found in the high metastatic potential group, and one in the low metastatic potential group (P < 0.05). The expression of *nm23-H1* mRNA was not correlated with some clinical factors, such as tumor size or the background liver disease.

CONCLUSION: The expression of *nm23-H1* mRNA is inversely correlated with HCC metastatic potential, and can be considered as an index which indicates the metastatic potential of HCC.

Key words: Liver neoplasms; *In situ* hybridization; Neoplasms metastasis; RNA; Messenger

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INTRODUCTION

nm23, a novel gene associated with low tumor metastatic potential, was isolated from a cDNA library of murine melanoma cells by Steeg *et al*[1] in 1988. Since then, a large number of investigations have been made by various methods. Although many studies have demonstrated that the *nm23* gene is differentially expressed in tumors with different metastatic potential[2-5]. Some researchers still have different viewpoints[6,7]. This preliminary study was designed to investigate the relationship between the expression of *nm23-H1* mRNA and the metastatic potential of human hepatocellular carcinoma (HCC).

MATERIALS AND METHODS

Materials

Twenty-four HCC specimens were obtained from the patients by operation. All the specimens contained cancer tissues and non-cancerous tissues. We divided the specimens into two groups according to the following criteria: (1) metastasis in portal lymph nodes; (2) ≥ 2 tumors in the liver; (3) cancerous emboli in the portal vein; and (4) the existence of satellite lesions. We named those meeting the criteria (1) or (2) and (3), or (3) and (4) high metastatic potential (n = 6); and the others formed the low metastatic potential group (n = 18). *Nm23-H1* cDNA was obtained as a gift from Rosengard. Dig-RNA labeling kits were purchased from Boehringer Mannheim Co., and *nm23-H1* cRNA probes were prepared according to the manufacturer's instructions.

Methods

In situ hybridization method was derived from several published papers with some modifications[8,9]. Frozen sections were fixed in 4% paraformaldehyde/PBS for 30 min at 4 ℃ and then rinsed sequentially with PBS, glycine/PBS, and Triton X-100/PBS. Hybridization buffer contained 2 ng/μL *nm23-H1* cRNA probes and the sections were hybridized in a 25 μL system. Optimal hybridization temperature

Table 1 Staining intensity of *nm23-H1* mRNA in the two groups

	Staining intensity		
	-	+	+ +
Low metastatic potential group	1	7	10
High metastatic potential group	3	3	0

$P < 0.05$, *vs* high metastatic potential group.

Table 2 Staining intensity of *nm23-H1* mRNA according to some clinical features

	Staining intensity		
	-	+	+ +
Liver tumor (s)			
Solitary	1	9	10
Multiple	3	1	0
Tumor size (cm)			
≤ 5	3	3	4
> 5	2	5	7
Associated liver disease			
Chronic hepatitis	1	2	5
Liver cirrhosis	6	5	11
Serum alpha-fetoprotein (ng/mL)			
≤ 100	2	6	7
> 100, ≤ 1000	2	3	3
> 1000, ≤ 10000	0	1	0

$P < 0.05$, compared with multiple group. $P > 0.05$

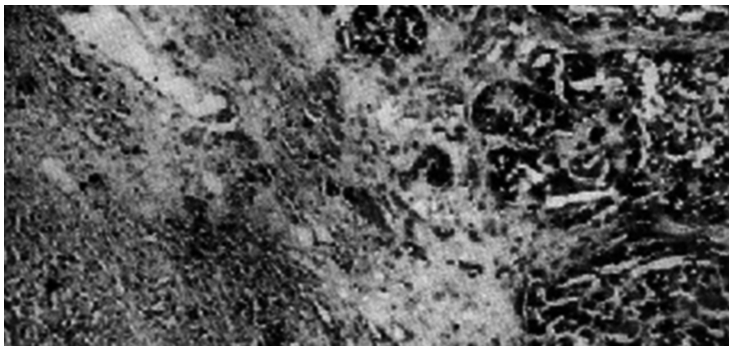

Figure 1 Staining intensity in hepatocellular carcinoma tissue (right panel) is higher than that in the adjacent nontumorous liver tissue (left panel). (Magnification, × 100)

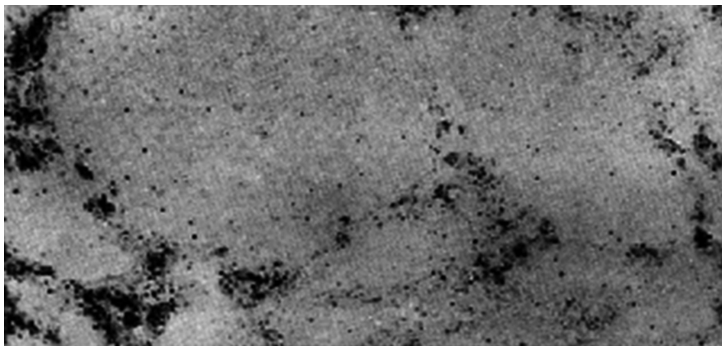

Figure 2 Cancerous nodules showing negative staining. (Magnification, × 100)

was about 50 ℃. As a negative control, the sections were hybridized with the sense probe or incubated with hybridization buffer only. To confirm the reactivity of the probes to tissue RNAs, the sections were digested with RNase (100 μL/mL) at 37 ℃ before hybridization. Statistical analyses were conducted by Ridit test. Results were considered significantly different when *P*-values were less than 0.05.

RESULTS

The positive results of *in situ* hybridization showed blue granules or masses in the cytoplasm. Staining intensity was graded as follows: (-), staining less intense than in adjacent non-tumorous tissue; (+), staining intensity similar to or slightly more intense than that in adjacent non-tumorous tissue; (++), staining more intense than in adjacent non-tumorous tissue (Figures 1-3). The results are shown in Tables 1 and 2. The *nm23-H1* mRNA expression in the high metastatic potential group differed significantly from that of the low metastatic potential group ($P < 0.05$).

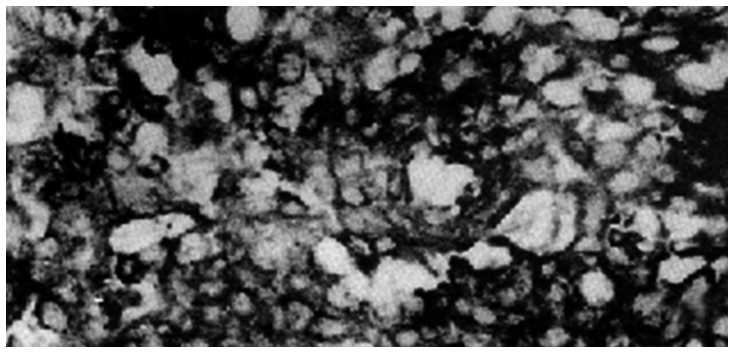

Figure 3 The positive signal of nm23-H1 mRNA is mainly distributed in the cytoplasm. (Magnification, × 200)

DISCUSSION

Many investigations have demonstrated that the *nm23* gene is closely related with tumor metastasis[4]. It has been widely accepted that *nm23* is the best candidate for a metastasis suppressor gene. The results of this study also showed that the expression of *nm23-H1* mRNA was inversely correlated with HCC metastatic potential. Rosengard found that nm23 protein existed in the cytoplasm and nucleus, but did not explain the specific functions of nm23 protein in different sites. Our study indicated that *nm23-H1* mRNA expression mainly existed in the cytoplasm. *Nm23-H1* cDNA probe was used in our study. Labeling kits used were DIG-DNA Labeling and Detecting kits (Boehringer Mannheim). The results of using *nm23-H1* cDNA are far from satisfaction. Possible reasons may be: (1) the sensitivity of detecting mRNA using cDNA probe is low; and (2) the quantity of *nm23-H1* mRNA is very small. In conclusion, our study shows an inverse relationship between the expression of *nm23-H1* mRNA and the metastatic potential of human HCC. The results of this paper warrant further studies with more cases and more detailed clinical features of each case. Our current efforts are focused on the detection of NDPA-A subunit, a product of *nm23-H1* gene, whose relationship with HCC metastasis deserves further studies.

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