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Brief Reports

Transient expression and antigenic characterization of HBsAg of HBV nt551 A to G mutant *

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INTRODUCTION

From the late 80s, there have been increasing number of reports on hepatitis B (HB) patients with atypical HBV serological markers, some of them even lack any HBV immunological markers. Analysis of the HBV in those patients demonstrated mutants. Mutations could be found within the C, S, P and X genes[1]. The most important S gene mutants are those which affect the antigenicity of HBsAg α determinant (HBsAg amino acid residue 124 to 147). There are several reports on HBV S gene mutants affecting amino acid position 126, 144 and 145 of HBsAg[1-3]. We have described another HBV mutant with a point mutation at nt551 (A to G) of HBV genome, leading to a substitution of Met to Val at amino acid re sidue 133 of HBsAg[4]. In this study, we investigated the antigenicity of the mutant HBsAg by different mAb.

MATERIALS AND METHODS

The recombinant bacteriophage pM13T was constructed and the mutant HBsAg coding region was sequenced (GenBank accession number AF052576)[4]. Construction of the mutant HBsAg expression plasmid pSHBsT and transfection of COS7 cell followed the reference[1]. The reactivity of the expressed HBsAg protein to mAb was detected by using a solid RIA kit (Beijing Atomic Energy Institute, China).

RESULTS

The wild HBsAg[1] and mutant HBsAg were expressed under the regulation of SV40 early promoter in COS7 cell in a transient fashion. A mAb against HBsAg *d* determinant (anti-*d*), S4 (Shanghai Institute of Biological Products, China), was used for the quantitation of the expressed HBsAg proteins. After a series of dilution and detection, both HBsAg preparations were adjusted to a concentration of 2.1 μg/L.

Three different mAb against HBsAg-α determinant (anti-α), A6, A11 and S17, from different manufacturers were selected to characterize the binding activity of the expressed HBsAgs. Under the condition of the same concentration of HBsAg proteins determined by anti-*d*, the reactivity of the mutant HBsAg to three anti-α mAb was weaker than that of the wild HBsAg, as shown in Table 1. The result implied that the Met to Val s ubstitution at amino acid position 133 of HBsAg resulted in the alteration of the antigenicity.

Table 1 Detection of the reactivity of the expressed HBsAg to anti-α mAb by radioimmunoassay*

Anti-a mAb	pSHBs(133Met)	pSHBsT(133Val)
A6	1118(5.82)	774(3.93)
A11	932(4.80)	744(3.76)
S17	945(4.87)	630(3.14)

*Counter per minute (cpm), the number in the parentheses is P / N-value. According to the solid RIA kit producer's instructions, P / N = (sample cmp-background) / (negative control cpm-background). Untrans fected cells were used as negative control, average cpm was 240. Blank polystyrene beads were used as background, average cpm 58. P / N ≥ 2.10 is cons id ered to be positive reactivity. The more the P / N value, the stronger the r eactivity.

DISCUSSION

Since Carman *et al*[3] described the HBV immune escape mutant in 1990, many researchers have reported that HBV DNA mutations are related to immune escape, but most of which are limited to the detection by PCR amplification and direct nucleotide sequencing. So far, only the mutant HBsAg with substitution of Ile to Ser at aa126[1], HBsAg with substitution of Asp to Ala at aa144[2], and HBsAg with substitution of Gly to Arg at aa145[3] were

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characterized in detail. Because the mutation of A to G at nt551 affect the HBsAg α determinant, it was likely that the mutation could cause immune escape[4]. This study showed that the mutation resulted in decreased reactivity of the HBsAg to anti-α monoclonal antibodies, confirming the hypothesis that the HBV is a new immune escape mutant.

The finding of HBV immune escape mutant has caused attention from scientists all over the world. Some experts recommended that it is worth considering to add mutant immunogen (HBsAg) into the future hepatitis B vaccine. But it is very important to know what mutants are immune escape ones and prevalent ones. A mutation specific PCR (msPCR) method for detecting the mutation at nt551 of HBV genome was established and the investigation and survey of the mutant among child and adult patients is in progress.

REFERENCES

1 Fang DX, Gan RB, Zhang Q, Li ZQ, Duan SC, Yin Z. Transient expression and antigenic characterization of HBsAg 126Ser of hepatitis B virus aa126 Ile to Ser mutant. *Chin J Virol*, 1998;14:1-9
2 Ni F, Fang D, Gan R, Li Z, Duan S, Xu Z. A new immune escape mutant of hepatitis B virus with an Asp to Ala substitution in aa144 of the envelope major protein. *Res Virol*, 1995;146:397-407
3 Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E. Vac-cine-induced escape mutant of hepatitis B virus. *Lancet*, 1990;336: 325-329
4 Fang DX, Lin HJ, Li FQ, Zhou ZX, Tan WG, Wang YL. Nucleotide sequencing of the S gene of a new hepatitis B virus immune escape mutant. *Chin J Microbiol Immunol*, in press.

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