

MASTER'S THESIS

Hepatitis B virus and single nucleotide polymorphisms

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**Hepatitis B Virus
and
Single Nucleotide Polymorphisms**

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for the degree of

Master of Philosophy

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Abstract

Hepatitis B is a major disease which causes serious public health problems worldwide. Hepatitis B virus (HBV) genome is composed of four open reading frames (ORFs), i.e. core C, surface S, polymerase P and X gene. The development of point mutations or single nucleotide polymorphisms (SNPs) on these genes during chronic HBV infection is associated with liver cirrhosis and hepatocellular carcinoma (HCC). Therefore, high throughput and simultaneous screening for these mutations is highly advocated for monitoring the disease development. Firstly, arrayed primer extension (APEX) was applied for the detection of HBV SNPs at the pre-C/BCP region. APEX was optimized for simultaneous detection of 8 SNPs in the pre-C/BCP region. The Pre-C/BCP regions of HBV from 36 HBV infected patients were amplified by PCR. After purification and fragmentation, the short single-stranded HBV DNA fragments were allowed to hybridize with the oligonucleotides corresponding to the SNPs immobilized on glass slides, followed by the incorporation of different fluorescently labeled dideoxynucleotides. This allows fast and unequivocal discriminations between wild type and mutant genotypes with high dideoxynucleotide incorporation efficiency, sensitivity and specificity. The coexistence of both genotypes was also detected, which was undetected by DNA sequencing. Then experience on APEX was further extended to identify the SNPs present in C, P, S and X gene of HBV genome. Thirty common SNPs were genotyped in 33 HBV carriers. The optimized APEX conditions were used. DNA sequencing was also involved for method validation. Again, high signal-to-noise ratios, sensitivities and specificities were obtained on most SNPs. The ability to identify the co-existence of wild-type and mutant genotype in one SNP indicating that APEX is a reliable HBV SNPs genotyping platform. By prevalence study on the 33 HBV infected patients, the most

frequent SNPs in the HBV genome are present in the pre-C/BCP region. Secondly, another technology, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used as an alternative tool to detect the 8 common SNPs present in pre-C/BCP region of HBV. By designing specific genotyping primers with known molecular masses, one dideoxynucleotide complementary to the SNPs could be extended unambiguously at the 3' end of the primers by DNA polymerase. The primer extension products were subjected to MALDI-TOF MS for mass analysis. The genotypes of each SNP were differentiated according to the mass differences between the extended and non-extended primers. The capability of MALDI-TOF MS to differentiate intrinsic molecular mass differences of dideoxynucleotides and high signal-to-noise ratio allowed unequivocal SNPs genotyping. In this study, APEX and MALDI-TOF MS were developed as fast, routine and non-radioactive platforms for HBV SNP genotyping. These platforms enable large-scale and diagnostic analysis, which is a possible alternative genotyping method to DNA sequencing. The application of APEX and MALDI-TOF MS as simultaneous and high-throughput genotyping platforms for HBV SNPs genotyping may be beneficial to the development of treatment strategies for this expanding chronic hepatitis B patient population. These technologies may also be extended to other SNP-based applications.

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