



Title	Effects of functional transient receptor potential channels on proliferation and migration in human cardiac c-kit+ cells
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Genetic analysis of occult hepatitis B virus infection

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Introduction: Occult hepatitis B infection (OBI) is characterised by absence of serum HBsAg and persistence of hepatitis B virus (HBV)-DNA in serum or liver. The HBV-DNA levels are usually extremely low. The mechanisms underlying OBI remain to be clarified. To determine if specific sequence variations of HBV genome may be associated with OBI, we used rolling circle amplification (RCA) method to overcome the difficulties in amplification of the full-length HBV genome (3.25 kb) from occult samples.

Methods: A total of 185 serum samples and 60 liver biopsies from subjects with OBI were analysed. Ten serum samples from subjects with overt HBV infection were used as controls.

Results: Twenty-two full-length HBV genomes were amplified from subjects with OBI and 10 from overt samples. The full-length HBV DNA was sequenced and aligned. Generally, occult HBV cases showed higher genetic changes than overt controls and sequence variations led to coding amino acid changes occurs through the viral genomes. We found point mutations and deletions led to abolishment of PreS2 start codon (ATG) in four occult samples that disrupt PreS2 protein synthesis. In addition, mutations turned 16 amino acid codons to stop codons that resulted in early termination of viral protein synthesis in 11/22 (50%) occult samples and 2/10 (20%) overt samples. We next explored the effect of mutation unique to OBI by studying the level of transcription activity driven by preS1 and preS2/S promoters, and core promoter using luciferase assays. The promoter activity on preS1 and S constructs were relatively similar to overt controls. However, the transcription activity of core promoter constructs with mutation at nucleotide 1677 and 1726 where transcription factor binds was only 72% and 40% of the control overt constructs, respectively.

Conclusion: Although some unique genetic changes were only detected in occult HBV samples, the findings from this study do not readily distinguish occult HBV from overt HBV.

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Effects of functional transient receptor potential channels on proliferation and migration in human cardiac c-kit⁺ cells

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Introduction: Human adult c-kit⁺ cardiac stem cell are characterised by the expression of c-kit in the absence of lineage markers such as Nkx2.5. They are self-renewing, clonogenic and multipotent, giving rise to a minimum of three differentiated cell types: myocytes, smooth muscle, and endothelial vascular cells. These cells, although not specifically programmed for myocardial differentiation, have been shown to improve cardiac function in a myocardial injury/reconstitution assay. However, cell biology is not fully understood. The present study was to investigate the expression of transient receptor potential (TRP) channels in human cardiac c-kit⁺ cells, and their role in regulating migration and proliferation.

Methods: Whole-cell patch voltage-clamp, RT-PCR, and Western blot approaches were used to determine functional expression of TRP channels in cultured human cardiac c-kit⁺ cells. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Wound healing and transwell assay were applied to observe the effect of the TRP channels on cell migration. Cell proliferation assay was made with MTT and ³H-thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La³⁺. Removal of Mg²⁺ of pipette solution or bath solution induced a Mg²⁺-sensitive current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. RT-PCR revealed significant mRNA expression of TRPC1, TRPC3, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. ShRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression. Interestingly, TRPV2-shRNA and TRPM7-shRNA significantly reduced proliferation of human cardiac c-kit⁺ cells. Migration of human cardiac c-kit⁺ cells was reduced by TRPV2-shRNA, TRPV4-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/3/4, TRPV1/2/4, and TRPM7 are present in human cardiac c-kit⁺ cells. TRPV2, TRPV4, and TRPM7 channels participate in regulating migration and proliferation.