

## HBV CORE PROMOTER MUTANTS ACTIVATE PI3K/AKT SIGNALLING IN CELL CULTURE, PROMOTING HEPATOCARCINOGENESIS

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**Background/Aims:** Hepatitis B virus (HBV) evolves during chronic HBV infection to assist evasion of host defences. Mutations in the core promoter (CP) region, which overlaps with the HBx gene, increase liver inflammation and are associated with increased risk of liver cancer, although the mechanisms are not clear. Most previous studies on CP mutations have focused on HBV genotypes B and C, the most prevalent genotypes in Asia, but little is known about CP mutations in HBV genotype D infection, which is increasingly common in Australia and is the focus of this study.

**Methods:** We engineered unique 1.2 mer HBV genotype D clones with changes in the CP region that correspond to G1764T/C1766G (TG double mutation) +/- G1757A. We expressed wild-type and mutant clones in Huh-7 cells by parallel transfection and supertransfection. HBV replication was confirmed by measuring HBsAg, HBcAg and Southern blotting. The effect of CP mutations on cell cycle progression and PI3K/Akt signalling was determined by western blot analysis.

**Results:** HBcAg expression was similar following transfection with wild or mutant clones. It remained stable after passaging, but doubled after super-transfection with wild or mutant clones, suggesting that infected cells are permissive to mixed infection. HBsAg was stably expressed in cells transfected with wild clones, but not mutant clones. When cells containing wild-HBV were supertransfected with mutant clones we observed increased phosphorylation of Akt (pAkt), with downstream activation of mTOR (4-EBP1) and MAPK (P38). Increased expression of atypical PKC isozyme PKC-iota was also observed. A potential role for PKC-iota in Akt signalling is under investigation. We observed reduced P21 expression with mutant transfection, but no effect on expression of other cell cycle proteins.

**Conclusions:** HBV clones containing HBx/CP mutations up-regulate PI3K/Akt signalling in our model of chronic HBV infection, potentially promoting liver cancer progression.