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論文審査担当者	主査： 岡本 尚 副査： 城 卓志, 田中 靖人

BACKGROUND &AIMS:

Entecavir (ETV) is approved for the first-line treatment of chronic hepatitis B virus (HBV) infections due to a high genetic barrier, but the virus can acquire resistance to the drug. This requires lamivudine resistance mutations (LAMr) and at least one additional mutation. Here, we characterized two novel mutations, rtI163V and rtA186T, associated with viral breakthrough (VBT) in an ETV-refractory patient.

METHODS:

HBV from an ETV-refractory patient was sequenced before ETV treatment and after VBT. Newly-identified mutations (rtI163T and rtA186T) were inserted into a replication-competent clone by mutagenesis. Clones were analyzed for replication efficacy and susceptibility to ETV in vitro. Chimeric mice with human hepatocytes were inoculated with the patient's serum at VBT, and monitored for viral mutation pattern using a next-generation sequencing approach.

RESULTS:

The novel mutations, rtI163V and rtA186T were detected together with LAMr (rtL180M and rtM204V) at VBT. RtA186T plus LAMr reduced susceptibility to ETV as strongly as previously reported ETVr mutations rtS202G plus LAMr, resulting in more than 111.1-fold resistance compared with the wild-type clone. While rtI163V plus LAMr resulted in a 20.4-fold reduction. The novel ETVr mutations did not confer cross-resistance to ADV. RtA186T significantly reduced viral replication efficacy, while the rtI163V mutation rescued it. Interestingly, the viral mutation pattern in the chimeric mice indicated dominant (or selective) proliferation of a clone containing rtI163V and rtA186T mutations plus LAMr under ETV treatment. Three-dimensional docking simulation indicated that rtA186T reduced the binding affinity of the HBV polymerase to ETV.

CONCLUSIONS:

VBT in this ETV-refractory patient is attributable to the novel ETV resistance mutations rtI163V and rtA186T. RtA186T was apparently responsible for ETV resistance but the selection of a clone with the double mutation plus LAMr suggests that rtI163V is required to sustain viral fitness. RtA186T and rtI163V should be closely monitored when chronic hepatitis B patients exhibit VBT during prolonged ETV treatment.