

ABSTRACT

The G1862T mutation in the bulge of the RNA encapsidation signal, in the precore region of hepatitis B virus, results in reduced expression of HBeAg and accumulation of the HBeAg precursor in the endoplasmic reticulum (ER)/Golgi apparatus of the cell. This accumulation can disturb the functioning of the ER and lead to the ER stress response that can affect various cellular pathways, in turn affecting cell viability. The aim of this study was to determine whether apoptosis or necrosis occurred when cultured Huh7 cells were transfected with a plasmid expressing the G1862T mutation. Plasmid constructs, with and without the G1862T mutation, were used to transfect cells. To differentiate between necrosis and apoptosis cells were stained with propidium iodide or YO-PRO-1[®], respectively. These were analyzed quantitatively using flow cytometry and qualitatively using confocal microscopy. Confocal microscopy, using monoclonal anti-HBe and the Hoechst stain, was performed to ensure that apoptosis was present as a result of the accumulation of the G1862T mutant HBeAg precursor. Caspase profiling was carried out using a fluorogenic-based assay. When cells were transfected with wild-type plasmid, necrosis predominated over apoptosis. Apoptosis predominated when the cells were transfected with the G1862T mutant plasmid. The highest levels of apoptosis occurred at 72 hours post-transfection. Confocal microscopy revealed the co-localization of aggregates of mutant HBeAg precursor with apoptotic nuclei. Transfection with G1862T mutant plasmids resulted in significant differences in the expression of caspase 3, 8, and 9 relative to the wild-type, at 48 and 72 hours post-transfection. The accumulation of the G1862T mutant HBeAg precursor, in the ER/ Golgi compartment, leads to apoptosis and affects the levels of caspase expression.