The gamma-glutamylcysteine synthetase (γ-GCS) mainly regulates de novo synthesis of glutathione (GSH) in mammalian cells and plays a central role in the antioxidant capacity of cells. It has been reported that drug-induced hepatotoxicity is partly caused by glutathione depletion. Therefore, a GSH-deficient animal model would be useful for the prediction of drug-induced hepatotoxicity. γ-GCS is a heterodimer consisting of a light regulatory subunit (GCSl) and a heavy catalytic subunit (GCSh) and catalyzes the rate-limiting step of GSH biosynthesis. The purpose of the present study is to construct a GSH-deficient cell line and experimental animal model for the prediction of human hepatotoxicity. An adenovirus vector with short hairpin RNA against rat GCSh (AdGCSh-shRNA) was used to knockdown the GCSh. H4IIE cells, rat liver hepatocarcinoma, were infected with AdGCSh-shRNA that efficiently decreased the endogenous levels of mRNA, the protein of GCSh and the GSH concentration. In the CYP3A4-over-expressing H4IIE cells by the treatment with a recombinant adenovirus encoding CYP3A4, the cytotoxicity of troglitazone was increased by the infection with AdGCSh-shRNA as well as by the treatment with L-buthionine (S,R)-sulfoximine (BSO), an inhibitor of glutathione production. In conclusion, the inhibition of γ-Gcs expression by AdGCSh-shRNA was similar to that by BSO in CYP3A4-overexpressing H4IIE cells, suggesting that the present method would be useful to evaluate the cytotoxicity caused by CYP3A4 mediated bioactivation. In addition, an in vivo experiment in rat using AdGCSh-shRNA will be demonstrated.