Cloning and prokaryotic expression of rat homolog of Serpina3n and its expression change during liver regeneration


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ABSTRACT. A strikingly upregulated expressed sequence tag was screened from regenerating rat liver at 8 h in a 0-4-8-12 h short-interval successive partial hepatectomy model from a previous study. In the present study, a full-length open reading frame (ORF) corresponding to this expressed sequence tag was predicted through electronic cloning and was subsequently cloned from an 8-h rat regenerating liver and deposited in GenBank (accession No. HM448398). Sequence analysis of HM448398 and the predicted ORF revealed that the two ORFs may be different transcripts of a gene. The sequence of HM448398 was highly homologous to that of rat Serpina3n, suggesting that it may be a homolog of Serpina3n. The pGEX-2TK prokaryotic expression vector for this ORF was constructed, and the result of sodium dodecyl sulfate polyacrylamide gel electrophoresis manifested that the recombinant expression vector could express the glutathione-S-transferase-fused rat homolog of Serpina3n in an insoluble form in BL21. The target fusion
protein was purified with affinity chromatography and was used as antigen to immunize rabbits for the production of polyclonal antibodies. Immunohistochemistry and real-time reverse transcription polymerase chain reaction analysis revealed that the gene was highly expressed in the priming and termination phases of liver regeneration. These findings lay a solid foundation for further study of roles of HM448398 using knock-in and RNA interference methods during liver regeneration.

**Key words:** Homologue of Serpina3n; Prokaryotic expression; Liver regeneration; Electronic clone; Expression change