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Intestine specific regulation of pig CMP-N-acetylneuraminic acid hydroxylase gene (pcmah) is specifically controlled by Sp1 binding sites on its promoter: 5'pcmah-1 containing exon 1a and common ORF region (exon 2 to exon 14) is intestine specific

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NeuGc is acted as an immune rejection antigen in pig to human xenotransplantation, as it is called as a non-Gal xenoantigen, a next xenoantigen to overcome after elimination of major Gal xenoantigen by knocking out the α-1,3-galactosyltransferase in the pig to human xenotransplantation. In the previous study, we isolated two promoter regions of P1 and P2, which are responsible for transcriptional regulation and located on upstream regions of the two alternative transcripts of 5'pcmah-1 and pcmah-2, respectively. Among them, promoter P2 was demonstrated to be responsible for basal house-keeping expression of the gene (BBRC). However, since the intestine tissues is important for the selective expression of the gene in responses to the pathogenic infection in pigs, the intestine specific regulatory mechanism of the gene promoter is the best interest of the pig NeuGc biosynthesis. Then, it is in this study reported that the 5'pcmah-1 promoter containing exon 1a and common ORF region (exon 2 to exon 14) is intestine specific in the pig. From the luciferase reporter assays using serial construction of each deleted promoter, it was demonstrated that promoter P1-1600 region relative to upstream region of 5'pcmah-1 is preferentially active in IPI-2I cells than in PK15 cells, corresponding with both mRNA expression patterns. Both promoters lack TATA box, but contain two Sp1 binding sites overlapped in the P1-260. Each mutation of Sp1 binding sites resulted in the reduction of luciferase activities in P1-542, indicating that in the proximal promoter region, Sp1 binding sites are crucial to regulate the intestine specific level of pcmah expression in the IPI-2I cells. In addition, the treatment with mithramycin A (25 nM to 100 nM) decreased the luciferase activity of P1 promoter in a dose-dependent manner. EMSA analysis revealed that the probes containing each Sp1 binding site bind to Sp1 and Sp3. Taken together, the results indicate that Sp1/3, or Sp1 bind to their putative binding sites on the P1 promoter regions of pcmah gene and positively regulate the promoter activity in pig cells.

Biography

Sun-Hyung Ha graduated from Biotechnology Department, DongA University, Busan, Korea in 2014. Presently, he is a master-doctor integrated coursed student at the Molecular and Cellular Glycobiology Lab, Department of Biological Science, Sungkyunkwan University, Suwon under supervisor Prof Cheorl-Ho Kim. His research interest is Glycan structure and biosynthesis of glycoproteins, glycoconjugates and glycolipids.

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