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Comparison of the Regulatory Regions of the α1,3Galactosyltransferase Gene Between Murine and Porcine Species

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THE major epitope (Galα1,3Gal) on pig endothelial cells that is recognized by naturally occurring antibodies in humans is a product of an enzyme called α1,3galactosyltransferase (α1,3GT), the gene for which is functional in pigs but not in Old World monkeys, apes, and humans.[1] In contrast, the functional analogue of α1,3GT in human is an enzyme called α1,2fucosyltransfetase (HT), the product of which (H-antigen) is expressed on the cell surface.[2] Using the murine H2Kb promoter construct, transgenic mice expressing H-antigen have been produced with relative ease.[3,4] However, the same is not true for pigs,[5–7] suggesting that the promoters are distinct in the two species. As the first step in clarifying whether any differences exist in the regulation of the α1,3GT gene in the two species, we already reported the regulatory regions in pig.[8] Here we report the isolation and characterization of the α1,3GT gene promoter regions and the total genomic organization in mice.

MATERIALS AND METHODS

To identify the 5′ and 3′ ends of α1,3GT gene transcripts, 5′- and 3′-RACE procedures were performed using the Marathon cDNA Amplification Kit (Clontech) with the spleen poly A^+ RNA of Balb/C adult male as template. To identify exon–intron boundaries or 5′- and 3′-flanking region of the transcripts, Murine Genome-Walker libraries were constructed using the Universal Genome-Walker Library Kit (Clontech) with Balb/C genomic DNA. To evaluate the promoter activity, we used Dual-Luciferase Reporter Assay System (Promega). Fragment of 1280 bp upstream from the position −350 (A of start codon is assigned +1) was cloned into multi-cloning site of the luciferase gene of a luciferase reporter vector, pGL3-Basic, provided in the kit, termed pGL3/1280. The pGL3-Basic (promoter-less) was used for the comparison.

RESULTS

Nucleotide sequences of our 5′-RACE are longer by 56 bp than previously reported by Joziasse et al.[9] The relative intensity of luciferase activity by the pGL3/1280 construct was 15-fold higher than that of pGL3-Basic. These data indicate that the (1280 bp) fragment has promoter activity, and that our 5′-RACE result most likely represents the potential transcription initiation site (TIS). Our 3′-RACE revealed an extended 3′-UTR sequence 30 bp more than previously reported,[9] but no other 3′ UTR exon usage. The overall length of the transcript was 3537 bp, 86 bp longer than previously reported.[9]

An overall comparison of 5′-UTR of cDNA sequences of the α1,3GT gene in porcine (675 bp) and murine (501 bp) shows that the homology is observed only in the region of exon 2 (71.7%). Exon 3 observed in mice is not observed in pig. Murine exon 1 shows no homology with porcine exon 1.

DISCUSSION

We have identified the regulatory region of the murine and porcine in α1,3GT gene. The sequence analysis of the 5′-flanking region of exon 1 revealed that there is no homology between the two species. Interestingly, despite this divergence, the two species have in common multiple GC-box, SP1, AP2, and other consensus motifs without TATA-box or CAAT-box immediately upstream of the transcription initiation site. However, they differ in that a CpG island is observed around TIS of this gene in the porcine, but not in the murine, species.

Taken together, these findings suggest that the regulation of α1,3GT gene expression may vary between the two species even though the enzyme performs an identical function. This would have important physiological implications and provide a possible explanation for the observed differences in the expression of the H-antigen in transgenic mice and pigs. In general, the genes in vertebrates that have a CpG island tend to be transcribed in early stage of cell replication.[10] Thus, provision of a porcine promoter rather than an H2Kb mouse promoter as has been previously reported[6,7] is likely to be the optimal condition for the regulation of a porcine α1,3GT gene in the porcine cells or tissues of transgenic animals.

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Exon-intron boundaries, 5′-flanking region of exon 1, 3′-flanking region of exon 9, and full-length cDNA sequences, are available in GenBank (accession number: AF297606 to AF297615). Our great thanks to Ms Therese Libert and Jennifer Profozich for their technical assistance, and to Ms Terry Mangan for secretarial assistance.

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