

HUMAN GENETICS & GENETIC DISEASES

and

MOLECULAR MEDICINE & DIAGNOSTICS

Methylation level of HLA-E transgene in the xenogeneic tissues of transgenic pigs

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Statement of the Problem: Epigenetics involves studies of changes in gene expression not associated with changes at DNA sequence level. The best characterized epigenetic mechanism involved in control of gene expression is DNA methylation. Methylation of cytosines in CpG islands within promoters usually causes gene silencing. In transgenic animals we are interested not only in introduction of transgene but also in its activity. We have prepared transgenic pigs for xenotransplantation purposes with decreased recognition by human immune system by introduction of the human HLA-E gene under elongation factor 1 alpha promoter (EF-1 α). Activity of HLA-E transgene was already confirmed on molecular level and flow cytometry.

Aim: The purpose of this study was evaluation of specific methylation of HLA-E transgene.

Methodology & Theoretical Orientation: Genomic DNA was modified by bisulfite conversion and PCR products were analyzed using pyrosequencing method. The most important steps were DNA isolation and DNA bisulfite conversion. Pyrosequencing enabled quantification of the specific methylation level of transgene.

Findings & Conclusion: The total level of methylation of the HLA-E transgene promoter was approximately 90% (range 67-100%) and the HLA-E gene 73.79% (29-100%). The obtained results of the HLA-E transgene methylation were compared to the results of transgene expression, which showed that the level of transgene expression in transgenic animals was stable, regardless of pig generation. This suggests that level of methylation of transgene promoter does not affect directly its biological activity estimated by flow cytometry.

Biography

Marlena Szalata works in biotechnology field with the main emphasis on transgenesis of animals and plants. She is involved in purification and characterization of recombinant proteins obtained in prokaryotic and eukaryotic systems like plants and animal bioreactors. She has expertise in molecular diagnostics of human, animal and plants genes. Recently she is involved in analysis of methylation level of selected human, animal and plant genes.

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