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Methylation status of H19 and IGF2 genes in fibroblast cell lines of goat (Capra hircus) after serum starvation treatment

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Due to the high incidences of abnormalities and the inefficiency in generating goat kids through somatic cell nuclear transfer (SCNT), the development of a model system in goat to investigate potential problems is essentially required. In nuclear transfer, where genomic imprinting has been implicated as a major cause for these problems, epigenetic regulation of developmentally important genes may give us the clue regarding the probable reasons for the low efficiency observed in SCNT. H19 and IGF2 are the genes that are involved in determining the imprinting status of donor cell in SCNT experiments. The study was conducted to investigate the methylation status of CTCF III binding region upstream of H19 gene and a fragment of IGF-2 exon 10region, before and after reprogramming of fibroblast cells by serum starvation method. Fibroblast cells were cultured up to sixth passage and genomic DNA was extracted before and after reprogramming. Genomic DNA samples were then used to amplify 295 bp fragment of H19 CTCF III binding region and 455 bp fragment ofIGF-2 exon 10region. The nucleotide sequence in H19 and IGF2 gene fragments had 19 and 27CpG motifs, respectively. Genomic DNA samples were then treated with sodium bisulphite to analyse the methylation status of identified CpG motifs. The bisulphite converted genomic DNA was amplified by bisulphite sequencing primer (BSP) set. The amplified fragments of bisulphite converted genomic DNA samples of reprogrammed and non-reprogrammed cells were then sequenced. Variation in the sequences were obtained from bisulphite converted genomic DNA of reprogrammed and non-reprogrammed cells. The nucleotide sequence analysis of bisulphite converted cultured non-reprogrammed cells revealed methylation of 6 CpG motifs in H19 gene fragment. Thus, the level of methylation observed in the study for 295 bp gene fragment was about 31.5%. However, in reprogrammed cells the CpG motifs were found to be unmethylated. Therefore, a reduction in the level of methylation was observed in reprogrammed fibroblast cells after serum starvation for this gene fragment. However, the fragment of IGF-2 exon 10 under investigation was hypermethylated in the fibroblast cells of goat in the serum- starved (0.5% Foetal Bovine Serum) and serum-fed cells (10% Foetal Bovine Serum). The DMR identified in the present study in goat can be used as an epigenetic tag for the detection of aberrant methylation. This may therefore serve as a promising approach for the early detection of putative developmental failures associated with artificial reproductive technologies incaprine species.

Keywords: H19 gene, IGF2 gene, Epigenetic, Serum starvation, Methylation, Fibroblasts; fibroblasts; Capra hircus.

Biography

Sanjeev Singh has completed his Masters and Ph.D from Indian Veterinary Research Institute (Deemed University), Izatnagar, Bareilly, UP. Thereafter, he joined Biotechnology Centre, MPPCVV (formally JNKVV) Jabalpur, as an Assistant Professor in the year of 2004. Currently he is working as a Senior Scientist in the DNA Fingerprinting Unit, National Bureau of Animal Genetic Resources, Karnal, Haryana, India. He has published several papers in journals of international and national repute. His area of research includes molecular and epigenetics studies of goat, RNA interference studies in caprine cell lines and Characterization of livestock breeds using microsatellite markers.

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