Oct4, the Most Important Pluripotency Gene: Cloning, Characterization and Bioinformatics Analysis in Endangered Wild Cats; Bengal Tiger, Snow Leopard and Jaguar

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Abstract

Oct4 is the most important gene involved in the reprogramming process of somatic cells into a pluripotent state. This is the first comprehensive report of Oct4 coding sequence in Tiger, Leopard and Jaguar. Coding sequences of Oct4 were successfully amplified and cloned from Tiger, Leopard and Jaguar fibroblasts. Bioinformatics analysis revealed that although the sequences of coding regions are slightly different between cat, tiger and jaguar, however, the theoretical protein consist of 360 amino acids is completely identical between these species. Cat Oct4 protein has 94.44%, 94.17% and 83.33% sequence similarities compared to human, dog and mouse Oct4, respectively. In contrast to the exons, introns and 5’ region close to the exon1 are less conserved in three species. A phylogenetic tree was built using the neighbor-joining method based on the alignment of the coding sequences of Oct4 gene. In conclusion, we first describe the molecular cloning and bioinformatics analysis of tiger, leopard and jaguar Oct4 gene, would be helpful for generating induced pluripotent stem cells (iPSCs) from somatic cells by direct molecular reprogramming, which have exciting possibilities to contribute to real-life species preservation.

Keywords: Tiger; Jaguar; Leopard; Cloning; Oct4

Introduction

It is generally agreed that induced pluripotent stem cells (iPSCs) can be useful for preventing the extinction process of some highly endangered species [1–3]. During last few years a set of reprogramming factors including Oct4, Sox2, Klf4, c-Myc, Nanog and Lin28 were extensively studied due to the importance of iPSCs in regenerative medicine [4], livestock industry [5] and other aspects of life sciences. Among these factors, Oct4 is the one cannot be replaced by other family members in the reprogramming process of human [4], mouse [6] and ruminants [7] fibroblasts into iPSCs.

For the first time Takeda J, et al. [8] reported the cloning of Oct4 and its expression in adult human tissues. Also the cattle, buffalo, goat, cat and rabbit orthologs of Oct4 gene have been cloned and characterized [9–13]. These studies showed high sequence identities of Oct4 between orthologous genes at the nucleotide and amino acid level as well as genomic organization, gene localization and regulatory regions. Oct4 contains two protein domains namely POU and Homeobox domains, both of them have a high degree of amino acid sequence conservation between species. In addition, it is known that alternative splicing, as well as usage of alternative translation initiation codons, results in multiple isoforms in human. Moreover, a number of Oct4 pseudogenes are exist in the human genome.

We recently reported the generation of wild cats’ iPSCs by forced expression of human pluripotency factors in Bengal Tiger, Snow Leopard, Serval and Jaguar fibroblasts [3,14]. However, due to the lack of genome information on these species the exact sequence of Oct4 gene is not available yet. Here we report sequencing and bioinformatics analysis of Oct4 gene in Tiger, Leopard and Jaguar.

Material and Methods

Cell Culture and Nucleic Acid Purification

Mitotically inactivated mEF were plated in 6 cm culture dishes. The Fibroblasts (FP) medium containing DMEM (high glucose, Invitrogen) supplemented with 20% (v/v) HyClone serum, 0.5% penicillin/streptomycin was used to isolate adult fibroblasts from ear tissue of all wild cats [3,14]. Nucleic acid purification was done by using DNeasy Blood & Tissue Kit (QIAGEN Inc.) according to the manufacture instruction.

PCR, cloning and sequencing of Oct4 gene

To isolate the Oct4 coding sequence, the following oligonucleotide primers were designed by using primer-BLAST [15] based on the published domestic cat (Felis catus) genome sequence (Figure 1). By using Platinum Taq DNA polymerase (Invitrogen) two, about 1760 and 1500 bp, fragments were successfully amplified for Exon 1 and Exon 2–5, respectively (Figure 1). Amplified fragments were cloned into the pGEMt vector (Promega) and sequenced.

Bioinformatics Analysis

To determine intron/exon boundaries, the Tiger, Leopard and Jaguar Oct4 gene sequences were aligned to the cat genomic sequence using CLC Main workbench software (Version 5.5) due to the incompleteness of wild cat genomic information. Multiple sequence alignments were conducted in CLC Main Workbench program, and a phylogenetic tree was constructed using the neighbor-joining method. The accession numbers of Oct4 sequences used for alignment and for the phylogenetic tree are listed in table 1. The molecular weight and isoelectric point (pl) were calculated by Compute pi/Mw (http://us.expasy.org/tools/pi_tool.html). The domain structure of the putative protein was analyzed on the SMART (http://smart.embl.de/) server.

Results and Discussion

Cloning and Sequence Analysis of Oct4 Gene

Two fragments of 1760 and 1500 bp were obtained and cloned
Figure 1: Nucleotide sequences and location of the primers designed in this study to amplify the coding sequence of Oct4 gene is shown. Exons are depicted as connected yellow rectangles. Ref Seq is downloaded from NCBI (accession: NC_018727, region: complement (32571100...32576320)). Size of the PCR products and the annealing temperatures used in cycling conditions for each pair is indicated above the template region.

Table 1: Pairwise comparison and list of the various Oct4 orthologs coding sequences used in the construction of the phylogenetic tree to analyze the evolution of Tiger, Leopard and Jaguar Oct4.

<table>
<thead>
<tr>
<th>Oct4 Genomic Region</th>
<th>Primer</th>
<th>Oligonucleotide Sequences (5’ to 3’)</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>F1</td>
<td>CGTTGGGAGATGGGGMGAAA</td>
<td>1760</td>
</tr>
<tr>
<td></td>
<td>R1</td>
<td>ACCCTACATTTGGGCGATGG</td>
<td></td>
</tr>
<tr>
<td>Exons 2–5</td>
<td>F2</td>
<td>GCAGTCCCAGGACATCAAAAG</td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>TCAGTTTGAATGGCATGAGAGG</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Primer pairs used to amplify the Oct4 gene in Tiger, Leopard and Jaguar.

Analysis of Translated Amino Acid Sequence of Oct4

Based on the predicted open reading frame, the deduced Oct4 protein is a 360 amino acid single polypeptide with a predicted molecular mass of 38.59 kDa and an estimated isoelectric point of 5.85. Despite the small differences in the genomic sequences of Tiger, Leopard and Jaguar Oct4 gene, the translated proteins are identical with cat Oct4 protein. The 360 amino acid long protein is in agreement with the Oct4 orthologs in human, dog, cattle, pig, and monkey (GenBank accession numbers are listed in Table 2), however [11] reported a putative 272 amino acids protein based on a sequenced CDS of 819 bp of cat Oct4. This difference might be occurred due to the different methodologies were used for detection of Oct4 gene.

One POU domain at the position 138 to 212 (E-value: 3.599e-48) and one HOX domain at the position 230 to 292 (E-value: 1.343e-18) were predicted by the SMART site. POU proteins are eukaryotic transcription factors containing a bipartite DNA binding domain...
Figure 2: The 13 bp deletion close to exon1 of Jaguar Oct4 gene.

Figure 3: Phylogenetic tree of the Oct4 gene between wild cats (highlighted in green) and other species.
Phylogenetic Analysis of Oct4 Gene

The results of alignment analysis showed that the coding sequence of Oct4 gene has 52.45%–99.82% identity to the Oct4 gene of other selected species (Table 2). To study the evolutionary relationship between wild cats Oct4 gene and other selected species, a phylogenetic tree was constructed based on the alignment of coding sequences using the neighbor-joining method in CLC Main Workbench software. As shown in figure 3, cats Oct4 were located at the base of the tree, considered ancestors at the molecular level, and close to the Canis which belongs to Carnivores. The Oct4 genes of rodents clustered together, clearly separated from Primates (Human and Monkey), with high bootstrap probability which agrees with the known fact that they are belong to Euarchontoglires. These results are in agreement with the mammal’s phylogenetic tree reported by [21].

Conclusion

In conclusion, we isolated the Tiger, Leopard and Jaguar Oct4 gene and used various bioinformatics tools to analyze the gene and predicted protein sequences. The information that was obtained provides an important support for conducting future studies on induced pluripotent stem cells.

Conflict of Interest

All the authors declared that they have no conflict of interest.

References


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