After the above analysis, particular gene expression profiles can be obtained for specific genes, or for groups of particular genes. Various gene expression patterns are shown in Figures 20 (a and b).

Figure 20 (a) depicts the gene expression for cytokine signalling in the immune system and innate immunity. It can be seen that these genes are the most over represented reactomes in OriP/EBNA1 induced cells when compared to closed linear DNA (dbDNA) mediated iPSC generation. The most significantly over-represented transcripts in OriP/EBNA1 iPSC compared to closed linear DNA induced iPSC were then analysed using Reactome pathway analysis.

Figure 20 (b) depicts the reactome sub-categorisation. Results show that Interferon alpha, beta and gamma signalling are the most strongly over-represented in OriP/EBNA1 induced cells when compared to closed linear DNA (dbDNA) mediated iPSC generation. Interleukin and NF-KB inflammatory signalling are also over-represented.

Example 10 : Quantitative RT-PCR

Quantitative RT-PCR was performed on iPSCs induced by either standard OriP/EBNA1 vectors or closed linear DNA. In particular, genes associated with interferon signalling, the innate immune system and inflammatory markers were investigated, or transcripts representing mesendoderm formation and early endoderm. Total RNA was extracted from the cells using standard methods.

The results found were are follows:

Figure 21 (a to g) depicts the results of a quantitative RT-PCR and comparison of Interferon signalling in OriP/EBNA1-induced versus closed linear DNA (dbDNA) – induced iPSC. Total RNA was extracted from cells generated using OriP/EBNA1 episomal plasmids or closed linear DNA vectors. All innate IFN signalling-associated transcripts were elevated in OriP/EBNA-iPSC compared to dbDNA-iPSC.

Figure 22 (a and b) depicts the results of a quantitative RT-PCR and comparison of inflammatory markers in OriP/EBNA1 -induced versus closed linear DNA (dbDNA) – induced iPSC. Total RNA was extracted from iPSC generated using OriP/EBNA1 episomal plasmids or closed linear DNA vectors. HMOX1 (a marker of oxidative stress) and NFKB1 (a marker of inflammation) are upregulated in OriP/EBNA1-iPSC compared to closed linear DNA-iPSC.

Figure 24 (a to d): depicts the upregulation of markers of differentiation in OriP/EBNA1-iPSC compared to closed linear DNA-iPSC (doggybone /dbDNA). Quantitative RT-PCR was used to evaluate transcripts representing mesendoderm formation and early endoderm as markers of early differentiation. In all instances there was increased markers of early differentiation in OriP/EBNA1-iPSC compared to closed linear DNA(dbDNA)-iPSC cultured under steady state conditions. Furthermore, the inventors noted increased expression of CDKN1A (p21), a cell cycle inhibitor, in OriP/EBNA1-iPSC compared to closed linear DNA-iPSC. CDKN1A-mediated inhibition of proliferation is a further indicator of differentiation of pluripotent stem cells.

Claims

1. An *in vitro* use of a closed linear DNA vector encoding one or more reprogramming factors for producing induced pluripotent stem cells from somatic cells, wherein said vector lacks sequences for chromosomal scaffold attachment.

2. An *in vitro* method of producing induced pluripotent stem cells (iPSCs) comprising introducing one or more closed linear vector(s) encoding one or more reprogramming factors into a population of somatic cells, and culturing said cells to effect expression of the one or more reprogramming factor(s), wherein said vector(s) lacks sequences for chromosomal scaffold attachment.

3. The use of claim 1 or the method of claim 2 wherein two or more closed linear DNA vectors are provided, each encoding one or more different reprogramming factors.

4. The use or method of any preceding claim wherein said reprogramming factor is selected from any one or more of: Oct 3/4, Sox2, Sox1, Sox3, Sox15, Sox18, Klf1, Klf2, Klf4, Klf5, c-myc, L-myc, and N-myc, NANOG, and/or LIN28.

5. The use or method of any preceding claim wherein said closed linear DNA vector lacks any sequence for the knockdown of p53.

6. The use or method of any preceding claim wherein said reprogramming factors are operably linked to one or more promoters, preferably operably linked to the same promoter on the closed linear DNA vector.

7. The use or method of any preceding claim wherein said closed linear DNA vector lacks one or more of:

(i) bacterial CpG motifs;

(ii) a bacterial origin of replication; and/or

(iii) antibiotic resistance genes.

8. The use or method of any preceding claim wherein the closed linear DNA vector is introduced into a mature somatic cell.

9. The use or method of any preceding claim wherein said closed linear DNA vector is introduced via transfection, optionally via nucleofection.

10. The use or method of claim 9 wherein the cell is cultured for around 30 days after transfection or nucleofection prior to reprogramming being complete, preferably 28 to 32 days.

11. The use or method of any preceding claim wherein said cells are human.