

THE ESTABLISHMENT OF FINNISH HUMAN EMBRYONIC STEM CELL LINES

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Introduction

The controlled differentiation and genetic manipulation of human ES (hES) cells *in vitro* and the transplantation of the differentiated ES cell derivatives could be used as potential therapy for several degenerative diseases. Also, hES cells could be used as a perfect model for studying human development from blastocyst stage on. At the moment only few hES cell lines have been established and fully characterized. More lines are required in order to confirm the reproducibility of results obtained so far. Even more importantly, large numbers of hES cell lines may be needed in order to establish tissue-typed cell banks for future cell transplantation purposes. Finland is among the few countries where generation of new hESC lines is currently possible.

Aims

1) To generate new hES lines. 2) To find new more specific markers for undifferentiated hES cells

Materials and Methods

*A total of 287 zygote or cleavage stage embryos were thawed. Of the survived frozen-thawed 233 embryos 68 (29%) blastocysts were formed (table 1) and 33 inner cell mass (ICM) isolations were performed.

*ES cells (figure 1.) were cultured on human feeder cells (CRL-2429, ATCC Mananas, USA) in serum-free medium (Knockout™ D-MEM; GibcoBRL, Life Technologies); supplemented with 2mM L-Glutamin (Sigma) 20% serum Replacement (GibcoBRL) , 1% non-essential aminoacids (GibcoBRL), 0.1mM beta-mercaptoethanol (Gibco BRL), 1x ITSF (Sigma) and 4ng/ml bFGF (Sigma)).

*The HESC were characterized by RT-PCR and immunohistochemistry for wellknown markers of undifferentiated ES cell phenotype. HLA-typing was done from DNA.

*Approximately 200 000 undifferentiated cells were transplanted into immunodeficient mice testes to confirm teratoma formation.

*Embryonic bodies (EB) were formed from overgrown hESC colonies in suspension culture (figure 2). Further differentiation was induced by plating EBs on gelatinized dishes.



Fig 1. A normal ES cell colony



Fig 2. Embryonic bodies in suspension

Table 1. All thawed embryos

	Zygote	Day 2	Day 3	Total
Number of embryos	36	244	7	287
Degenerated embryos	8 (8/36 = 22%)	43 (43/244 = 18%)	3 (3/7 = 43 %)	54 (17 %)
d 5 blc	2	20	0	22
d 6 blc	7	39	0	46
d5+d6 blc (%)	9 (9/36=25 %)	59 (59/244=24 %)	0	68 (68/244=29 %)

Results

•Four continuously growing cell lines (FES 21, 22, 29 and 30) have been achieved.

•They express the stem cell markers Oct-4, FGF-4, SSEA-4, Tra 1-60, Tra 1-81, Nanog and Pumilio-2 (Figure 3) and karyotypes are normal.

•All cell lines spontaneously differentiate to neuron-like cells in high density culture, and result in teratoma formation after transplantation in nude mice.

•Pumilio 2 (PUM2) appears not to be as specific as nanog for undifferentiated hES cells (Figure 4).

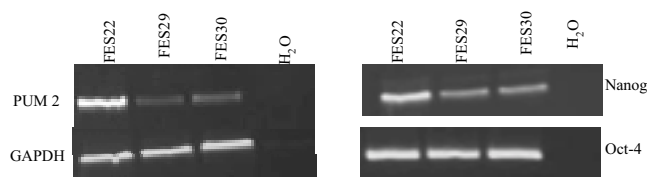


Fig 3. Undifferentiated ES cells mRNA expression were analyzed by RT-PCR

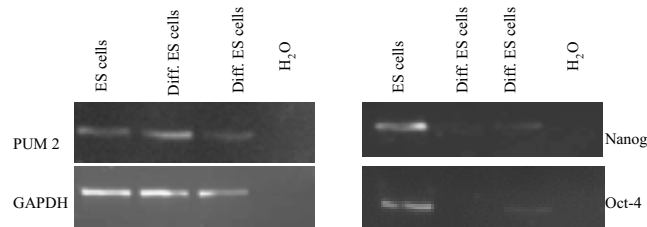


Fig 4. Differentiated ES cells mRNA expression were analyzed by RT-PCR

Conclusions

*The expression of ES-cell markers, the spontaneous *in vitro* differentiation and teratoma formation in immunodeficient mice verify the pluripotent nature of the four new hES cell lines.

*PUM 2, a protein required to maintain germ line stem cells in *Drosophila* and *C. elegans*, is expressed in the FES cells lines.

*However, PUM2 expression is not immediately downregulated at the time of hES cell differentiation and hence, may not be considered as a specific marker for undifferentiated hESC.

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