MAINTENANCE OF HUMAN EMBRYONIC STEM CELLS IN A PLURIPOTENT STATE

Factors and signalling pathways required for hESC self-renewal, pluripotency maintenance and differentiation of hESCs into cell types from all three germ layers

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ABSTRACT

Human embryonic stem cells (**hESCs**) are derived from the inner cell mass of blastocyst stage pre-implantation embryo and have the unlimited capacity for self-renewal and the ability to maintain its pluripotent state that enables them to differentiate into cell types from all three germ layers: ectoderm, mesoderm and endoderm. The endodermal derivation is crucial for treatment of diabetes, liver and pulmonary diseases because it allows the generation of pancreatic islet progenitors, hepatocytes, and lung alveolar cells. The mesodermal derivation of hESCs could be the key in cancer treatment and in immunological therapies because it allows the generation of dendritic, functional T and NK cells and cardiomyocytes. Finally, the ectodermal derivation of hESCs displays a potential use in cell replacement therapies in several neurodegenerative diseases because it allows the generation of dopaminergic, cholinergic and motor neurons.

1 MOLECULAR AND CELLULAR PROPERTIES OF hESCs

Key features

Unlimited capacity for self-renewal

Capacity to differentiate into cell types from all three germ layers

Cell markers

TRA-1-60 TRA-1-81

SSEA-3 SSEA-4

High alkaline phosphatase expression

High levels of telomerase activity

Culture medium

MEFs

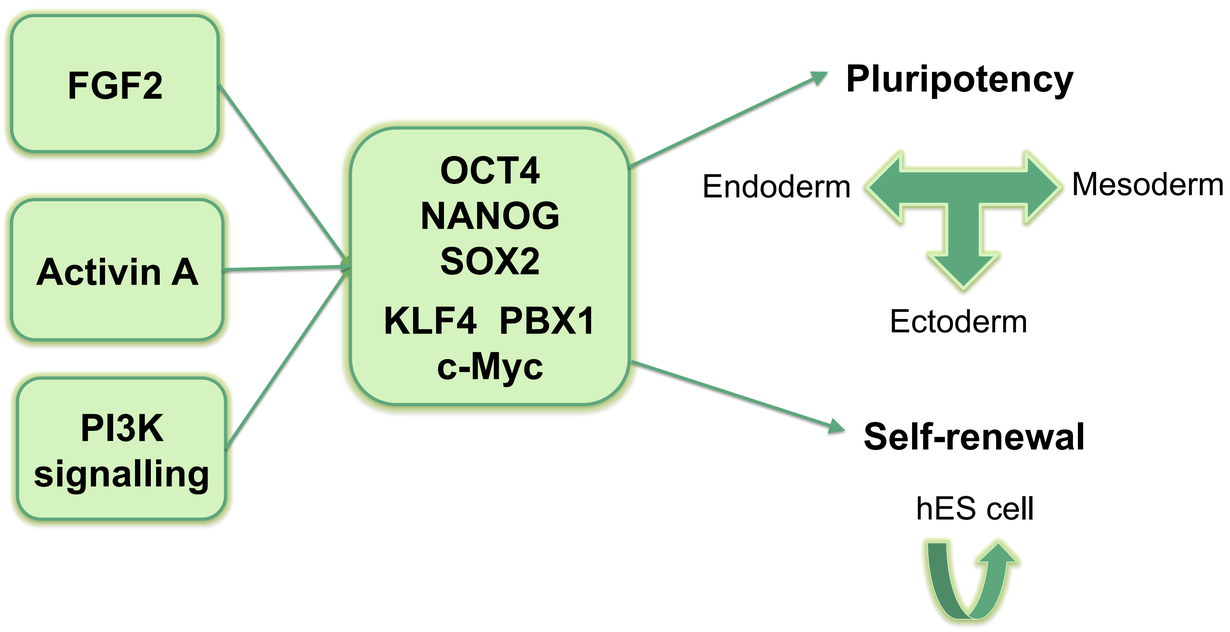
Xenobiotic-free culture medias and feeder-free culture systems

Determination of hESCs pluripotency

Induction of teratomas formation

Formation of embryoid bodies

2 SIGNALLING PATHWAYS REQUIRED FOR hESC SELF-RENEWAL AND PLURIPOTENCY



3 DIFFERENTIATION OF hESCs INTO CELL TYPES FROM ALL THREE GERM LAYERS

	Cell type	Method	Specific factors and/or conditions
Endodermal derivation of hESCs	Hepatocytes	Differentiation of hESC into DE, followed by sequential exposure to differentiation factors	Low serum media Collagen I matrix FGF, BMP4 Hepatocyte growth factor Oncostatin M Dexamethasone
	Pancreatic islet progenitors		Activin A, Wnt3A Keratinocyte growth factor/FGF7 Retinoic acid Cyclopamine Noggin
	Lung alveolar cells	Genetic modification of hESCs followed by spontaneous differentiation	Recombinant keratinocyte growth factor

	Cell type	Method	Specific factors and/or conditions
Mesodermal derivation of hESCs	Dendritic cells	Human embryoid body formation	Serum-free conditions BMP4
	T and NK cells	Co-culture with stromal cells	Co-culture with stromal cells (M210-B4)
	Chondrocytes	Human embryoid body formation	High density micromass of dissociated EB BMP2
		Directed differentiation on 3D scaffolds	Co-culture with mature chondrocytes
	Cardiomyocytes	Human embryoid body formation	Serum-free conditions FGF2
		Directed differentiation	Activin A BMP4

	Cell type	Method	Specific factors and/or conditions
Ectodermal derivation of hESCs	Dopaminergic neurons	Co-culture with stromal cells	FGF8 Shh
		Formation of neural rosettes	FGF8 Shh
	Cholinergic neurons	Formation of neurospheres	Shh FGF8 BMP9
	Motor neurons	Formation of neural rosettes	Shh Retinoic acid
	Oligodendrocytes	Directed differentiation	B27, thyroid hormone retinoic acid, FGF2 EGF, insulin
	Retinal pigment epithelium		Serum-free conditions Nicotinamide, Activin A

4 CONCLUSIONS

- ✓ The hESCs could be the key in regenerative medicine but first of all it is necessary develop efficient methods to isolate them from earlier stages without destroying the embryo, and, thereby, without compromising ethical and political restrictions.
- ✓ There is a need for develop culture medias chemically defined and free of animal products to enable hESCs maintain its pluripotent state and its capacity for self-renewal without compromising their chromosomal stability.
- ✓ The current protocols to differentiated hESCs into specific cell types are still not completely safe for using them as a human clinical trial, it is necessary first produce a large and uniform population of pluripotent cells, without teratomas formation and stable genetically, even in prolonged culture.

5 REFERENCES

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