Stem Cells

-cells in our bodies which have the ability to differentiate into different tissue types

-ability to harness the potential of stem cells to differentiate into a specific tissue type will clearly have great benefits in regenerative medicine

-only the use of epithelial stem cells in skin and cornea grafting, and transplantation of haematopoietic stem cells for treating certain blood disorders are clinically established

-developing in vitro culture protocols and establishing characterization standards of stem cells, both embryonic and adult, are therefore vital.

Embryonic Stem Cells

-Human embryonic stem cells (hESCs) are derived from the inner cell masses (ICMs) of blastocysts

-They are pluripotent cells, having the capacity to self-renew and differentiate in vitro and in vivo into a wide variety of tissues exhibiting the characteristics of all three germ layers.

-Pluripotent, can form 3 layers of ectoderm, mesoderm and endoderm

-Infinite life span due to expression of telomerase

-Form teratoma when injected into immune-compromised mouse

-Clump to form an embryoid with cells that differentiate spontaneously

-Directed differentiation is possible with addition of specific growth factors that direct cell down a specific pathway of differentiation

In vivo differentiation by teratoma formation in SCID mice

-hESCs are capable of differentiating into tissues of all three germ-layers in vivo.

-This is observed as teratoma formation after transplantation.

-Inject more than 2 x 10^6 hESCs into a testis of SCID male mouse

-Harvest teratoma after 3-5 months

-Fix teratoma in 4% paraformaldehyde and embed in paraffin

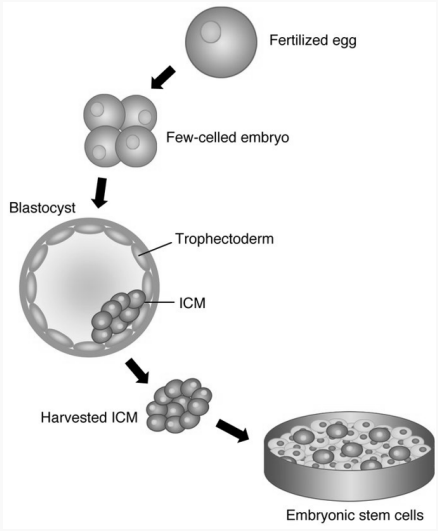
Embryonic Stem Cells (cont)

-Examine teratoma histologically

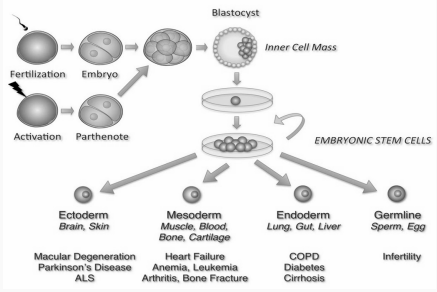
Teratoma: A type of germ cell tumor that may contain several different types of tissue, such as hair, muscle, and bone. A rare type of tumor that can contain fully developed tissues and organs, including hair, teeth, muscle, and bone. Teratomas are most common in the tailbone, ovaries, and testicles, but can occur elsewhere in the body. Teratomas can appear in newborns, children, or adults. They're more common in females.

SCID: Severe combined immunodeficiency

Embryonic Stem Cells Source



Embryonic Stem Cells



Adult Stem Cells

-Undifferentiated cells found among differentiated cell

-Multipotent, divide to form progenitor or precursor cell e.g. epithelial cell and fibroblast

-Epithelial cell and fibroblast are proliferative in culture, therefore will dominate the culture

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Adult Stem Cells (cont)

-Some are self-renewable such as hematopoietic stem cell of bone marrow

-Others are not self-renewable with finite life span such as mesenchymal stem cell

-Fetal stem cell has more potential or capacity for proliferation but less adaptable to adult environment

Normal & Transformed Animal Cells

Normal Animal Cells	Transformed Animal Cells
-Diploid chromosome number with no gross chromosomal damage	-Chromosome fragmentation is often associated with transformation – aneuploidy
-Anchorage dependent	-May lose anchorage-dependence – cell could grow as suspension
-Finite life span	-Infinite growth capacity – also known as continuous cell line
-Non-malignant/ cancerous	-Transformed animal cells are not necessarily cancerous
-Telomerase activity absent	-Telomerase activity present
	-May lose contact inhibition – cell could grow as multilayer
	-Form tumour when injected into immuno-compromised mice

Cancerous cells display malignancy in addition to the characteristics of transformed cells

Cell Line Identification

-to prevent cross-contaminated cell being used for research

(google)-provides researchers with confidence that their cell lines are correctly identified, and not cross contaminated with other cells

Methods

1. Karyotyping
2. Isozyme pattern
3. Antibody labelling

Cell Line Identification (cont)

4. DNA fingerprinting
5. Telomerase test

1. Karyotyping

-nucleoprotein is partially digested by trypsin, and the Giemsa dye produces a characteristic pattern of G bands

-banding patterns are characteristic for each chromosome pair and permit recognition

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-determines the species of origin and determine the extent of gross chromosomal changes in the line

-cell lines with abnormal karyotype are also used if they continue to perform normal function

-Karyotype is affected by the growth conditions used, the way in which the cells are subcultured and whether or not the cells are frozen.

Giemsa-Banding

-dye gives chromosome a stripped appearance

-it stains the regions of DNA that are rich in adenine (A) and thymine (T) base pairs

-regions that stain as dark G bands replicate late in S phase of cell cycle & contain more condensed chromatin

-regions that stain as light G bands replicate early in S phase & contain less condensed chromatin

Protocol

1. Incubate hESCs in hESC culture media with 0.1 µg/ml colcemid for 3 hrs at 37°C in a humidified C02 incubator
2. Harvest single cells using trypsin-EDTA and treat them with a hypotonic solution (1 % sodium citrate buffer) at 37°C for 30 mins
3. Fix with methanol and acetic acid (3:1 , v/v)
4. Spread hESCs onto a glass slide, dry and identify chromosomes by G banding

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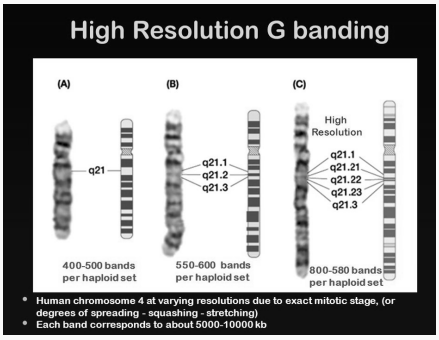
G-Banding



2. Isozyme/Isoenzyme Analysis

-based on the existence of enzymes with similar or identical specificity, but different molecular structure

-study the patterns of migration of isoenzymes present in cell lysates following electrophoresis using agarose gels under non-denaturing conditions

-patterns obtained are species specific and therefore are used as quality control and authentication procedures to confirm species of origin of material

-Specific activity stains are used to develop a banding pattern of isozymes (zymogram), which is characteristic of a particular cell line.

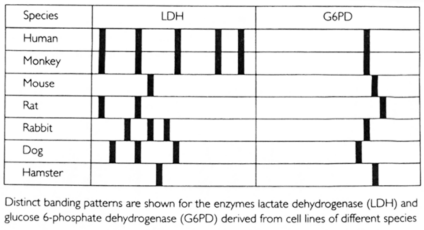
-By using several enzymes the distinguishing features of a cell line are established.

-These features can often distinguish cell lines even if derived from the same species.

-This is a more rapid technique compared to karyotyping and requires smaller cell samples.

-Enzymes: Glucose 6-phosphate dehydrogenase (G6PD), lactate dehydrogenase (LDH), nucleoside phosphorylase, alkaline phosphatase, creatine kinase, glucokinase, hexokinase, glutathione S-transferase (GST)

Isozyme/Isoenzyme Analysis



Distinct banding patterns are shown for the enzymes lactate dehydrogenase (LDH) and glucose 6-phosphate dehydrogenase (G6PD) derived from cell lines of different species

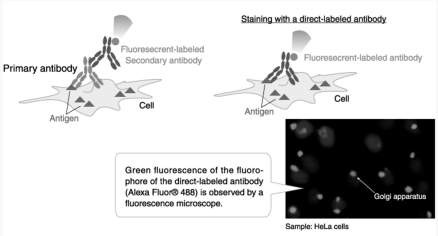
3. Labeled Antibodies

-use of a fluorescent-labeled antibody specific for a membrane antigen

-The antibody, is conjugated to a suitable fluorescent compound, such as fluorescein.

-The conjugate will bind specifically to the outer membrane of the chosen cells which can be identified by fluorescence microscopy or by a fluorescence-activated cell sorter

Labeled Antibodies



4. DNA Fingerprinting

-A unique DNA 'fingerprint' can be developed for a particular cell line.

-DNA fingerprint results from the fragmentation pattern produced by digestion of cellular DNA with restriction endonucleases

-The resulting restriction fragment digest is separated by electrophoresis

-Radioactive probes are then used to hybridize to specific restriction fragments which can be highlighted by autoradiography.

-results in a characteristic 'bar-code' pattern

-The most useful probes for this purpose are those that hybridize to 'mini-satellite' DNA

-These are repetitive nucleotide sequences of varying length found throughout the genomic DNA.

-Certain restriction enzymes (for example, HinfI) are used because they are known to cut DNA within the minisatellite regions.

-The length and distribution of the resulting minisatellite DNA fragments are unique to individuals and hence can be used for identification, including cell line identification.

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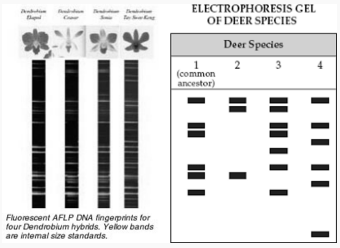
DNA Fingerprinting



Fluorescent AFLP DNA fingerprints for four Dendrobium hybrids. Yellow bands are internal size standards.

5. Telomerase Test

-Initial tumor formation can occur in the absence of telomerase, tumor maintenance requires telomere stabilization, most often accomplished through the activation of telomerase.

-If telomerase expression is detected, it is transformed cell line or cancer cell line

-If telomerase expression is not detected, it is deer liver normal cell line

-Telomerase extend telomeres

-A telomere is the end of a chromosome. Telomeres are made of repetitive sequences of non-coding DNA that protect the chromosome from damage. Each time a cell divides, the telomeres become shorter. Eventually, the telomeres become so short that the cell can no longer divide.

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