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The diagram illustrates the differentiation pathway for human pluripotent stem cells into germ cells, showing the progression from pluripotent stem cells to various germ cell stages and finally to spermatozoa.

Pluripotent Stem Cells (represented by a cluster of blue cells) differentiate into **Epiblast-like cells** (represented by a cluster of grey cells) under the influence of **Activin a** and **Fgf2**.

Epiblast-like cells differentiate into **Primordial germ cell-like cells** (represented by a cluster of blue cells) under the influence of **Bmp4**, **Scf**, **Lif**, and **Egf**.

Primordial germ cell-like cells differentiate into **Oocyte-like cells** (represented by two large blue cells) and **Follicle-like structures** (represented by two smaller blue cells) under the influence of **Insulin**, **Egf**, and **hCG**.

Oocyte-like cells and **Follicle-like structures** differentiate into **Primordial germ cell-like cells** (represented by a cluster of blue cells) under the influence of **BMP4**, **BMP7**, and **BMP8B**, along with the **+ transduction of DAZ genes**.

Primordial germ cell-like cells differentiate into **Primordial germ cell-like cells** (represented by a cluster of blue cells) under the influence of **Sort**, **Integrin-β3+**, and **Ssea1+**.

Primordial germ cell-like cells differentiate into **Spermatozoa** (represented by a sperm cell) under the influence of **Transplantation into testes**.

The diagram illustrates the differentiation pathways for various cell types from Pluripotent Stem Cells (PSCs). The pathways are as follows:

- Epidermis:** PSCs are cultured on an artificial matrix with Ascorbic acid and BMP4 to differentiate into Keratinocytes, which then become Epidermis.
- Retinal pigment epithelium:** PSCs are cultured with Nicotinamide and Activin A to differentiate into Retinal pigment epithelium.
- Ectoderm:** PSCs are cultured with DKK1, WNT, NODAL, and Activin to differentiate into Ectoderm. Ectoderm can further differentiate into:
 - Photoreceptors:** Ectoderm is cultured with RA and Taurine to become Photoreceptors.
 - Otic hair cells:** Ectoderm is cultured with Fgf2 and Igf1 to become Otic hair cells.
 - Neural progenitors:** Ectoderm is cultured with FGF (FGF2, EGF) and BMP4 (inhibited by NOGGIN, LDN, Dorsomorphin) to become Neural progenitors.
- Neuroectoderm:** PSCs are cultured with PA6/MS-5 coculture or SFEBq culture to differentiate into Neuroectoderm. Neuroectoderm can further differentiate into:
 - Optic cup:** Neuroectoderm is cultured in 3D matrigel culture to become Optic cup (Neural retina (NR) and Retinal pigment epithelium (RPE)).
 - Cortical layers:** Neuroectoderm is cultured with INSULIN, TRANSFERIN, and Selenium to become Cortical layers.
- Neural Progenitors and Differentiation:** Neural progenitors can differentiate into various cell types:
 - Astrocytes:** Neural progenitors are cultured with CNTF to become Astrocytes.
 - Oligodendrocytes:** Neural progenitors are cultured with FGF2, SHH, and PDGF to become Oligodendrocytes.
 - GABA neurons:** Neural progenitors are cultured with SHH to become GABA neurons.
 - DA neurons:** Neural progenitors are cultured with RA and SHH to become DA neurons.
 - Spinal motor neurons:** Neural progenitors are cultured with KSR or adherent culture to become Spinal motor neurons.
 - Cortical neurons:** Neural progenitors are cultured with Retinoids and Sort p75+, HNK1+ to become Cortical neurons.
 - Neural crest stem cells:** Neural progenitors are cultured with FGF8 and SHH to become Neural crest stem cells.
- Sorting and Further Differentiation:** Neural progenitors and Neural crest stem cells are sorted by CD73+ and can further differentiate into:
 - Optic cup:** Neural progenitors are sorted by CD73+ and cultured in 3D matrigel culture to become Optic cup.
 - Cortical layers:** Neural progenitors are sorted by CD73+ and cultured with INSULIN, TRANSFERIN, and Selenium to become Cortical layers.

The diagram illustrates the differentiation of endoderm from pluripotent cells. It starts with a cluster of yellow spheres representing pluripotent cells, which are treated with BMP4, FGF2, EGF, and VEGF. These cells are then sorted for CXCR+ and CD117^{high} expression. The sorted cells can differentiate into multipotent endodermal progenitor cells or definitive endoderm. The definitive endoderm is further differentiated into various lineages: hepatic progenitors (via FGF10, RA, and inhibition of ACTIVIN/NODAL by SB431542), pancreatic progenitors (via FGF10, RA, SHH, and inhibition of TGFβ by A-83-01), hindgut endoderm (via WNT3A and FGF4), anterior endoderm (via inhibition of TGFβ by A-83-01 and inhibition of BMP4 by NOGGIN), and multipotent lung progenitors (via BMP4, FGF2, and WNTs). The hepatic progenitors further differentiate into hepatocytes (via HGF, FGF4, and EGF) or β cells (via IGF1, HGF, and Nicotinamide). The pancreatic progenitors further differentiate into β cells (via IGF1, HGF, and Nicotinamide) or intestinal tissue (via NOGGIN, inhibition of BMP4, and 3D matrigel culture). The hindgut endoderm further differentiates into intestinal tissue (via EGF and 3D matrigel culture).

Hematopoietic stem cell differentiation pathways:

- OP-9 coculture:**
 - From **Hematopoietic stem cell** to **Primitive streak mesoderm** (factors: BMP4, VEGF, SCF, TPO, FLT-3).
 - From **Hematopoietic stem cell** to **Mesenchymal stem/precursor cells** (factors: FGF2, PDGF-BB).
- Primitive streak mesoderm differentiation:**
 - To **Hemangioblast** (factors: BMP4, VEGF, SCF, TPO, FLT-3).
 - To **Hematopoietic progenitors** (factors: VEGF, IL-3, IL-6, IL-11, SCF).
- Hemangioblast differentiation:**
 - To **Erythropoietic cells** (factors: SCF, TPO, IL-3, FP6, EPO).
 - To **Hematopoietic progenitors**.
- Hematopoietic progenitors differentiation:**
 - To **Lymphoid progenitors** (via OP-9 or MS-5 coculture).
 - To **Monocyte/macrophage progenitors** (factors: M-CSF, RANKL).
- Monocyte/macrophage progenitors differentiation:**
 - To **Osteoclasts**.
- Mesenchymal stem/precursor cells differentiation:**
 - To **Adipogenic cells** (factors: DEXA, IBXT, Insulin).
 - To **Chondrogenic cells** (factors: BMP4, OP-9 coculture, DKK, WNT, VEGF).
 - To **Cardiovascular colony-forming cells** (factors: SB431542, TGFβ).
 - To **Endothelial cells** (factors: Fibronectin, EGM2).
 - To **Osteogenic cells** (factors: β-GP, DEXA, Ascorbic acid).
 - To **Skeletal myoblasts** (via N2-Serum).
 - To **Smooth muscle cells** (factors: VEGF, FGF2).
 - To **Cardiomyocytes** (factors: VEGF, FGF2).

hESC and hiPSC research is one of the most dynamic fields in modern biology, but cell-based clinical applications are currently limited by xeno contamination during the in vitro derivation and propagation phases. Thus, bridging the gap between research models and clinical applications requires the design and implementation of qualified protocols and operating processes. Xeno-free or animal-component-free media is an essential element in the development of regenerative stem cell therapies where implantation in humans is the desired outcome.

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