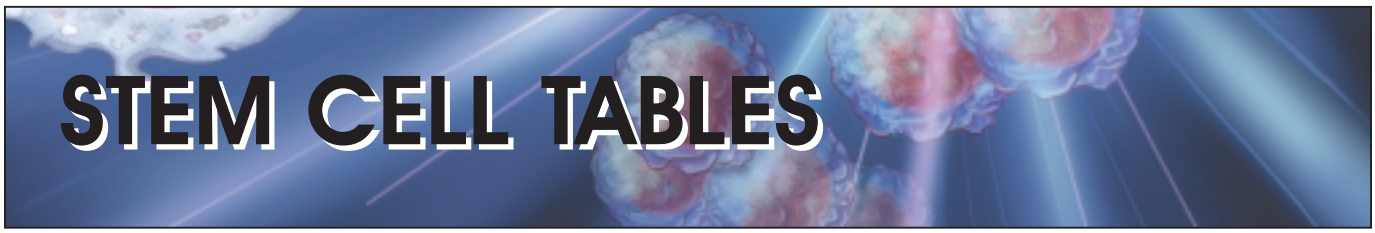
## APPENDIX D:



# STEM CELL TABLES

## COMPENDIUM OF SCIENTIFIC PUBLICATIONS REGARDING THE ISOLATION AND CHARACTERIZATION OF STEM CELLS

The following tables provide an overview of information about stem cells that have been derived from mice and humans. The tables summarize published research that characterizes cells that are capable of developing into cells of multiple germ layers (i.e., multipotent or pluripotent) or that can generate the differentiated cell types of another tissue (i.e., plasticity) such as a bone marrow cell becoming a neuronal cell. The tables do not include information about cells considered progenitor ore precursor cells or those that can proliferate without the demonstrated ability to generate cell types of other tissues.

The tables list the tissue from which the cells were derived, the types of cells that developed, the conditions under which differentiation occurred, the methods by which the cells were characterized, and the primary references for the information.

## Appendix D.i.
## Published Reports on Isolation and Differentiation of Mouse Stem Cells

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| Tissue | Cell Type | | | | |
| Bone marrow | Hematopoietic stem cell (HSC) | Cardiac muscle | Cardiac injury induced in mice<br>Injected labeled HSCs into injured heart | Measurement of green fluorescent protein (GFP) in regenerating cardiac cells<br>Measurement of cardiac-specific protein and gene expression<br>Cardiac-function tests | Orlic et al., 2001 |
| | HSC | Epithelial cells of the liver, skin, lung, esophagus, stomach, small and large intestine | Transplantation of HSCs into lethally irradiated female mice | Detection of antibodies to cellular and cell-surface proteins<br>Cell staining<br>Probing for Y chromosome-positive cells | Krause et al., 2001 |
| | HSC | Cholangiocyte<br>Hepatocyte | Purification of HSCs from bone marrow<br>Transplantation of HSCs into mice with liver-enzyme deficiency | Observation of normalized liver function and regenerating hepatocytes<br>Measurement of expression of hematopoietic and hepatic cell-surface proteins | Lagasse et al., 2000 |
| | HSC | Platelet<br>Red blood cell<br>White blood cell | Hematopoietic growth factors: interleukin-3, interleukin-6, granulocyte-colony stimulating factor, erythropoietin, and thrombopoietin | Detection of antibodies to cell-surface proteins<br>Colony-forming assays<br>Immunophenotyping | Spangrude et al., 1991<br>Morrison et al., 1995 |
| | HSC<br>Side population (SP) | Skeletal muscle | Lethal irradiation of female mice<br>Induced muscle injury<br>Purified bone marrow transplanted into mice | Measurement of dystrophin expression in regenerating muscle cells<br>Fluorescence-activated cell sorting (FACS)<br>Probing for Y chromosome-positive cells | Gussoni et al., 1999 |
| | Mesenchymal stem cell (MSC) | Adipocyte<br>Chondrocyte<br>Osteoblast<br>Tenocyte | Dexamethasone<br>Vitamin $D_3$<br>Bone morphogenetic protein-2 (BMP-2) | Detection of antibody binding to cell-surface proteins<br>Immunofluorescence | Friedenstein et al., 1976<br>Pereira et al., 1995<br>Prockop, 1997 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Bone marrow *(cont.)* | MSC | Astrocyte<br>Neuron | Injection of MSC into brain of immunocompromised neonatal mice | Detection of cell-surface markers by using antibodies and immunofluorescence | Kopen et al., 1999 |
| | MSC | Astrocyte<br>Neuron | Epidermal growth factor<br>Brain-derived neurotrophic factor<br>ß-mercaptoethanol<br>Retinoic acid | Immunofluorescence<br>Cell sorting | Sanchez-Ramos et al., 2000 |
| | MSC | Neuron | Stromal cells expanded as undifferentiated cells<br>ß-mercaptoethanol<br>Butylated hydroxyanisole | Detection of numerous neuron-specific proteins via staining | Woodbury et al., 2000 |
| | MSC | Skeletal muscle | 5-azacytidine and amphotericin B | Observation of myotubes<br>Staining for myocytes | Wakitani et al., 1995 |
| | MSC and/or HSC | Astrocyte<br>Microglia<br>Oligodendrocyte | Induced injury to neural tissue<br>Bone marrow transplantation | Detection of antibodies to cell-surface proteins | Eglitis and Mezey, 1997 |
| | MSC and/or HSC | Cardiac muscle | Bone marrow transplantation of 5-azacytidine-treated cells into mice with induced cardiac muscle injury | Cell staining for cardiac muscle proteins<br>Measurement of blood pressure<br>Electron microscopy<br>Observation of beating cells *in vitro*<br>Measurement of atrial natriuretic peptide<br>Staining cells for muscle proteins | Tomita et al., 1999<br>Makino et al., 1999 |
| | MSC and/or HSC | Hepatocyte | Suppression of liver cell proliferation<br>Induced injury to liver<br>Bone marrow transplantation | Staining cells<br>Antibody labeling of cell-surface markers | Taniguchi et al., 1996<br>Petersen et al., 1999<br>Theise et al., 2000 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Bone marrow *(cont.)* | MSC and/or HSC | Neuron | Induced neural tissue injury<br>Bone marrow transplantation into female mice | Detection of antibodies to cell-surface proteins<br>Probing for Y chromosome-containing neurons | Mezey et al., 2000<br>Brazelton et al., 2000 |
| | MSC, HSC, or side population (SP) | Cardiac muscle<br>Skeletal muscle | Lethal irradiation of mice<br>Bone marrow transplantation from normal male donor mice into mice with induced muscle degeneration | Probing for Y chromosome-containing muscle cells<br>Detection of expression of myoregulatory proteins | Bittner et al., 1999 |
| | MSC, HSC, or SP | Skeletal muscle | Induced muscle tissue injury<br>Transplantation of genetically marked bone marrow into immunodeficient mice | Histologic observation of muscle regeneration<br>Detection of antibodies to cell-surface proteins<br>Myogenic differentiation factor transcript expression | Ferrari et al., 1998 |
| | SP | Cardiomyocyte<br>Vascular endothelia | Transplanted into lethally irradiated mice with ischemic damage to cardiac tissue | Immunohistochemistry<br>Staining for cardiomyocte marker (alpha-actin) and endothelial marker (flt-1) | Jackson et al., 2001 |
| Brain | Neural stem cell (NSC) | Astrocyte<br>Neuron<br>Oligodendrocyte | Basic fibroblast growth factor<br>Epidermal growth factor | Detection of antibodies to neural cell-specific proteins | Reynolds et al., 1996<br>Doetsch et al., 1999<br>Johansson et al., 1999 |
| | NSC | Red blood cell<br>White blood cell | Transplantation of NSC into irradiated mice | Flow cytometry analysis<br>Genetic labeling assay<br>Detection of antibodies to cell surface proteins | Bjornson et al., 1999 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Brain *(cont.)* | NSC | Skeletal muscle | Transplantation of NSCs into mice<br>*In vitro* co-culture with myogenic cells | Observation of differentiated skeletal muscle cells<br>Analysis of muscle cell-specific proteins and gene expression | Galli et al., 2000 |
| Embryo-blastocyst inner-cell mass | Embryonic stem (ES) | Adipocyte | Retinoic acid<br>Insulin, T3 (thyroid hormone), and Leukemia inhibitory factor (LIF) | Observation of adipocyte differentiation<br>Measurement of adipocyte enzyme activity<br>Measurement of adipocyte-specific gene expression | Dani et al., 1997 |
| | ES | Astrocyte<br>Glial precursor<br>Oligodendrocyte | Cells cultured in neurogenic medium with basic fibroblast growth factor<br>Epidermal growth factor<br>Platelet-derived growth factor<br>Transplanted glial precursor cells into myelin-deficient mice | Observation of spinal cord remyelination<br>Electron microscopy<br>Antibodies to neural cell-specific proteins | Brustle et al., 1999 |
| | ES | Astrocyte<br>Midbrain neuron<br>Neural precursor<br>Neuron<br>Oligodendrocyte | Retinoic acid<br>Cell selection through transgene conferring drug resistance<br>Co-culture with stromal cells | Examination of cell morphology and neuron-specific markers<br>Cell-specific markers<br>Detection of dopamine production | Bain et al., 1995<br>Strubing et al., 1995<br>Li et al., 1998<br>Lee et al., 2000<br>Kawasaki et al., 2000 |
| | ES | Astrocyte<br>Neuron<br>Oligodendro-cyte | Retinoic acid | Observation of functional synapses<br>Measurement of neurotransmitters | Slager, et al., 1993<br>Gottlieb, et al., 1999 |
| | ES | Astrocyte<br>Oligodendrocyte | Retinoic acid<br>Fetal calf serum (10%)<br>ß-mercaptoethanol | Antibodies to neural cell-specific proteins<br>Cytochemistry | Fraichard et al., 1995 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Embryo-blastocyst inner-cell mass *(cont.)* | ES | Cardiac muscle<br>Skeletal muscle<br>Smooth muscle | Retinoic acid<br>Dimethyl sulfoxide<br>Transplantation of muscle cells into mice | Histology<br>Detection of cell-specific proteins<br>Cytochemistry | Dinsmore et al., 1996 |
| | ES | Cardiomyocyte | LIF, retinoic acid<br>Fibroblast feeder cells | Histology and observation of beating cardiomyocyte<br>Detection of specific cardiac cell-gene expression and cardiomyocyte surface proteins | Doetschman et al., 1985<br>Maltsev et al., 1993<br>Wobus et al., 1995 |
| | ES | Cardiomyocyte | LIF<br>Cell selection through genetic labeling of ES<br>Injection of ES into mouse heart | Detection of genetically labeled cardiomyocytes<br>Electrophysiological studies | Bader et al., 2000 |
| | ES | Cardiomyocyte | LIF<br>Purification of cardiomyocytes from ES culture by genetic labeling and selection | Observation of functional cardiomyocyte grafts in heart<br>Immunohistology | Klug et al., 1996 |
| | ES | Cardiomyocyte | Culture of ES with LIF<br>Selection of cardiomyocytes through genetic labeling<br>Injection of cardiomyocytes into mouse heart | Microscopy and cell-receptor studies<br>Observation of cardiomyocyte differentiation and contractility<br>Analysis of cardiomyocyte gene expression | Westfall et al., 1997 |
| | ES | Chondrocyte (cartilage-forming cell) | BMP-2 and BMP-4 | Staining of mature chondrocytes<br>Measurement of chondrocyte-specific gene expression and proteins | Kramer et al., 2000 |

***Appendix D.i.*** *(cont.)*

| Origin Tissue | Origin Cell Type | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| Embryo-blastocyst inner-cell mass *(cont.)* | ES | Dendritic (immune cell) | Culture on stromal cell layer<br>Interleukin-3<br>Granulocyte-macrophage stimulating factor | Immune-function assays<br>Immunophenotyping | Fairchild et al., 2000 |
| | ES | Embryoid bodies (EBs) consisting of structures that contain tissues of the three embryonic germ layers: endoderm, mesoderm, and ectoderm<br>Teratocarcinoma | ES cultured in suspension without feeder cell layer<br>Absence of LIF<br>Injection of ESs into mice | Observation of differentiation into multiple tissue types of the germ layers of blood, skeletal and cardiac muscle, primitive gastro-intestinal and neural tissue<br>Growth of tumor containing tissues from embryonic germ layer | Evans and Kaufman, 1981 |
| | ES | ES self–renewal | LIF<br>Culture on feeder cell layer | Observation of extensive ES proliferation and self-renewal | Evans and Kaufman, 1981 |
| | ES | Endothelial | Culture on collagen substrate<br>Hematopoietic growth factors<br>Semisolid media<br>EB implanted peritoneal cavity | Observation of capillary formation | Risau et al., 1988 |
| | ES | Endothelial<br>Smooth muscle<br>Vascular progenitor | Culture over collagen-IV matrix<br>Absence of LIF<br>Vascular endothelial growth factor | Electron microscopy: observation of endothelial and smooth muscle vascular structures<br>Detection of endothelial cell marker by immunochemistry<br>Detection of smooth muscle markers by immunochemistry | Yamashita et al., 2000 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Embryo-blastocyst inner-cell mass *(cont.)* | ES | HSC and erythroid | Interleukin-6<br>Absence of LIF and cell feeder layer<br>Culture on collagen substrate<br>Hematopoietic growth factors<br>Semisolid media<br>BMP-4 | Antibodies against surface markers<br>FACS<br>Immunophenotyping | Wiles and Keller, 1991<br>Johansson and Wiles, 1995<br>Perkins et al., 1998 |
| | ES | Keratinocyte (skin) | ß-mercaptoethanol<br>Implantation of ES cells in mice | Microscopy<br>Immunofluorescence<br>Observation of skin tissue differentiation<br>Measurement of keratin | Bagutti et al., 1996 |
| | ES | Lymphoid precursor<br>Lymphocyte | Culture of ES in low oxygen concentration (5%) without hematopoietic growth factors | Antibodies to lymphoid cell-surface proteins<br>Analysis of antibody production and lymphocyte receptors | Potocnik et al., 1994 |
| | ES | Macrophage | Interleukin-3 and macrophage colony stimulating factor | Immunophenotyping<br>Immune-function assays | Lieschke and Dunn, 1995 |
| | ES | Mast | Lethal mutations in ES cells<br>Culture of EBs in media containing interleukin-3, stem cell factor | Transplantation of cells into mast cell-deficient mice<br>Immunologic- and inflammation-function tests<br>Analysis of gene expression | Johansson and Wiles, 1995<br>Tsai et al., 2000 |
| | ES | Melanocyte | Dexamethasone<br>Stromal cell layer<br>Steel factor | Morphology studies<br>Reactivity to growth factors<br>Expression of melanogenic markers | Yamane et al., 1999 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Embryo-blastocyst inner-cell mass *(cont.)* | ES | Neuron | Expression of noggin cDNA in ES<br>Expression of neuronal determination gene<br>EB exposed to retinoic acid | Detection of antibodies to neuronal proteins | O'Shea, 1999 |
| | ES | Oligodendrocyte | Retinoic acid<br>Induced spinal cord injury<br>Transplantation of ES-derived cells into spinal cord of mice | Detection of remyelination in spinal cord<br>Antibodies to oligodendrocyte-specific proteins | Liu et al., 2000 |
| | ES | Osteoblast (bone cell) | Co-cultured with fetal mouse osteoblasts<br>Dexamethasone, retinoic acid, ascorbic acid, ß-glycerophosphate | Microscopy; observation of mineralized bone nodules<br>Histochemistry | Buttery et al., 2001 |
| | ES | Pancreatic | Insertion of insulin-gene promoter into ES | Antibodies to cellular proteins<br>Measurement of insulin, glucagon, somatostatin<br>Observation of islet-like organization of cells<br>Transplantation of cells into diabetic mice with resultant lowering of blood glucose | Soria et al., 2000 |
| | ES | Pancreatic islet-like | Serum-free media<br>Absence of feeder-cell layer<br>Basic fibroblast growth factor<br>Nicotinamide | Detection of antibodies to cellular and cell-surface proteins | Lumelsky et al., 2001 |
| | ES | Skeletal muscle | Overexpression of insulin-like growth factor-2 in ES through gene insertion<br>Dimethyl sulfoxide | Observation of myocyte differentiation<br>Measurement of myocyte-specific gene expression and proteins | Prelle et al., 2000 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Embryo-blastocyst inner-cell mass *(cont.)* | ES | Skeletal muscle | Transforming growth factor-beta and retinoic acid<br>ES co-culture with stromal cells<br>Fetal calf serum<br>ß-mercaptoethanol | Observation of myocyte differentiation<br>Detection of functional muscle cell receptors<br>Measurement of myocyte-specific gene expression | Slager et al., 1993<br>Rohwedel et al., 1994 |
| | ES | Smooth muscle | Retinoic acid and db-cAMP<br>Culture over collagen IV matrix<br>Vascular endothelial growth factor<br>Platelet-derived growth factor-BB | Electron microscopy observation of vascular structures<br>Detection of smooth muscle markers: SMA, CGA7 | Drab et al., 1997<br>Yamashita et al., 2000 |
| | ES | Smooth muscle | Platelet-derived growth factor | FACS<br>Detection of smooth muscle cell proteins | Hirashima et al., 1999 |
| | ES | White blood cell | Interleukin-3<br>Transplantation of ESs into lymphocyte-deficient mice | Measurement of lymphocyte-specific gene expression<br>Radioimmunoassay | Wiles and Keller, 1991 |
| | ES | White blood cell | Transplantation of ES cells into lymphocyte-deficient mice | Histology<br>Immunophenotyping<br>Antibodies to cell-specific proteins | Rathjen et al., 1998 |
| Gonadal ridge (fetal) | Embryonic primordial germ cell | Endoderm<br>Mesoderm<br>Ectoderm | "Reprogramming" primordial germ cells: culture of primordial germ cell with LIF, basic fibroblast growth factor and<br>Steel factor | Histology<br>Immunocytochemistry | Matsui et al., 1992 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Liver | HSC | HSC<br>All blood cell lineages | Enrichment of cell populations through immunoselection<br>Purification of $CD45^+$ liver cells<br>Selection of cells with HSC markers<br>Transplantation of HSCs into lethally irradiated mice | Colony-forming assays<br>Detection of *in vitro* growth of hematopoietic colonies by flow cytometry and cell sorting<br>Liver-derived cells reconstituted from bone marrow of transplanted mice<br>FACS | Taniguchi et al., 199 |
| Pancreas | Pancreatic ductal epithelial cell | Alpha, beta, and delta pancreatic islet | Stem cells isolated from prediabetic adult, nonobese mice<br>Cells cultured for an extensive period<br>Pancreatic cells transplanted into diabetic mice | Analysis of pancreatic cell gene expression and differentiation markers<br>Glucose challenge test *in vitro*<br>Observation of reversal of insulin-dependent diabetes in mice with transplants | Ramiya et al., 2000 |
| | Unselected pancreatic cells | Hepatocyte | Pancreatic cells transplanted into mice with liver-enzyme deficiency | Detection of normalized liver function in mice<br>Histological evidence of donor-derived hepatocytes | Wang et al., 2001 |
| Skeletal muscle | Muscle | Adipocyte | Long-chain fatty acids<br>Thiazolidinediones | Assays of adipocyte enzyme function<br>Observation of adipocyte differentiation<br>Detection of adipocyte-specific gene expression | Grimaldi et al., 1997 |
| | Muscle | Osteoclast and osteocyte<br>Osteoprogenitor | Exposure of donor cells to BMP-2<br>Retroviral transfection of cells with vector and transplantation into severe combined immunodeficient mice (SCID) | Detection of ectopic bone formation<br>Detection of muscle-derived cells<br>Co-localization with osteocalcin-producing cells in newly formed bone matrix | Bosch et al., 2000 |

***Appendix D.i.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Skeletal muscle *(cont.)* | Muscle Satellite | HSC<br>Myocyte precursor | Isolation of transcription factor Pax7 as a gene expressed specifically in satellite cell-derived myoblasts | Detection of $Pax7^{-/-}$ and $Pax7^{+}$ muscle cells in hematopoietic and myogenic cells | Seale et al., 2000 |
| | Muscle Satellite or SP | All blood cell lineages<br>HSC | Transplant of muscle-derived cells into lethally irradiated mice | Observation of engraftment of muscle cells in bone marrow<br>Antibodies to hematopoietic cell markers<br>FACS | Jackson et al., 1999<br>Gussoni et al., 1999 |
| | Satellite | Myocyte<br>Myocyte precursor | Induced tissue injury; mechanical and denervation stress<br>Transcription factor expression | Detection of myocyte progenitor and myocyte-specific proteins and mRNA transcripts | Megeney et al., 199 |
| Spinal cord | NSC | Astrocyte<br>Neuron<br>Oligodendrocyte | Basic fibroblast growth factor<br>Epidermal growth factor | Detection of antibodies to neural cell proteins | Weiss et al., 1996 |

## Appendix D.ii.
## Published Reports on Isolation and Differentiation of Human Fetal Tissue Germ Cells

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| Tissue | Cell Type | | | | |
| Gonadal ridge | Primordial germ cell | Embryoid bodies | SDEC line of embryoid body derived cells<br>transplanted into rats paralyzed with a virus induced motor neuron degeneration | Functional assessment of rat locomotion and righting ability (turning from supine to prone)<br>Histopathologic examination of motor axons<br>Immunohistochemistry of mature neurons: $NeuN^{+}$ and 68-kilodalton neurofilament | Kerr et al., 2001 |
| | Primordial germ cell | Embryoid bodies with neural cells, vascular endothelium, muscle cells, endodermal derivatives | Leukemia inhibitory factor,<br>Basic fibroblast growth factor | Clonal expression, polymerase chain reaction<br>Ethidium bromide fluorescence detection<br>Surface markers: 68-kilodalton neurofilament, neuron-specific enolase, tau, vimentin, human nestin, galactocerebroside, O4, SMI32 | Shamblott et al., 2001 |
| | Primordial germ cell | Embryoid bodies with three germ layers: endoderm, mesoderm, ectoderm | Leukemia inhibitory factor,<br>Basic fibroblast growth factor | Detection of surface markers: SSEA-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 | Shamblott et al., 1998 |

## Appendix D.iii.
## Published Reports on Isolation and Differentiation of Human Embryonic Stem Cells

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| Tissue | Cell Type | | | | |
| Human embryo (from *in vitro* fertilization (IVF)) | Blastocyst inner-cell mass | Ectoderm<br>Endoderm<br>Mesoderm<br>Neuronal progenitor cell | Leukemia inhibitory factor<br>Injection into severe combined immunodeficient (SCID) mice | Developed two lines (HES-1, HES-2)<br>Clonal expression<br>Polymerase chain reaction<br>Surface markers: SSEA-1, SSEA-4, TRA-1-60, GTCM-2 | Reubinoff et al., 2000 |
| | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Cardiomyocyte | Embryoid body formation (See Itskovitz-Eldor et al., 2000) | Visualization of contracting areas in embryoid bodies<br>Immunohistochemistry for cardiac myosin heavy chain, alpha-actinin, desmin, cardiac troponin I, and antinaturetic protein. | Assady et al., 2001 |
| | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Cardiomyocyte | Embryoid body formation | Polymerase chain reaction for cardiac-specific genes and transcription factors | Kehat et al., 2001 |
| | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Cardiomyocyte<br>Endoderm<br>Hematopoietic<br>Neuron | Leukemia inhibitory factor<br>Basic fibroblast growth factor<br>Collagenase or trypsin/EDTA to induce embryoid body | Clonal expression<br>Polymerase chain reaction<br>Surface markers: gamma-globin, 68-kilodalton neurofilament, alpha-fetoprotein, albumin | Itskovitz-Eldor et al., 2000 |
| | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Ectoderm: brain, skin, adrenal<br>Endoderm: liver, pancreas<br>Mesoderm: muscle, bone, kidney, urogenital, heart, hematopoietic, hematopoietic | Basic fibroblast growth factor, transforming growth factor beta 1, activin-A, bone morphogenic protein 4 hepatocyte growth factor, epidermal growth factor, beta nerve growth factor, retinoic acid | Clonal expression<br>Polymerase chain reaction<br>Surface markers | Schuldiner et al., 2000 |

***Appendix D.iii.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Human embryo (from *in vitro* fertilization (IVF) *(cont.)* | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Ectoderm: neural epithelium, embryonic ganglia, stratified squamous epithelium<br>Endoderm: gut epithelium<br>Mesoderm: cartilage, bone, smooth muscle, striated muscle | Injection of cell lines into severe combined immunodeficient mice<br>Leukemia inhibitory factor<br>Type IV collagenase | Surface markers: SSEA-3, SSEA-4, TRA-160, TRA-181, alkaline phosphatase<br>Radioimmunoassay detection: alpha-fetoprotein and human chorionic gonadotropin | Thomson et al., 1998 |
| | Blastocyst inner-cell mass (H9 clone line from Thomson et al., 1998) | Pancreatic beta cell | Embryoid body formation<br>(See Itskovitz-Eldor et al., 2000)<br>No leukemia inhibitory factor or basic fibroblast growth factor | Immunohistochemistry for insulin<br>Polymerase chain reaction for insulin, IPF1/PDX1, Ngn3, beta-actin, Glut-1, Glut-2, glucokinase, and Oct 4 | Assady et al., 2001 |

## Appendix D.iv.
## Published Reports on Isolation and Differentiation of Human Embryonic Carcinoma Stem Cells

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| Tissue | Cell Type | | | | |
| Terato-carci-noma | Embryonic carcinoma (EC) | Endodermal progenitor cell | Absence of feeder cell layer<br>Bone morphogenetic protein-2<br>Retinoic acid | Analysis of stem cell marker-gene transcription<br>Immunochemistry<br>Immunofluorescence | Roach et al., 1994<br>Pera and Herszfeld, 1998 |
| | EC | Neuron | EC transplanted into mouse brain | Observation of functional synapses<br>Immunochemistry | Trojanowski et al., 1993 |
| | EC | Glial<br>Neuron | Retinoic acid | Measurement of mRNA for GABA(A) receptor-chloride complex<br>Recording of whole-cell voltage-clamp measurements in differentiated cells in the presence of GABA(A) receptor antagonists and activators (bicuculline and flurazepam, respectively) | Reynolds et al., 1994 |
| | EC | Glial<br>Neuron | Retinoic acid | Detection of neurons with HNK-1 antibody<br>Measurement of acetylcholine synthesis and detection of high-affinity uptake sites for GABA | McBurney et al., 1988 |
| | EC | Neuron | Retinoic acid | Morphology and histology<br>Analysis of neuron-specific proteins | Andrews, 1984 |

***Appendix D.iv.*** *(cont.)*

| Origin | | Cell Types | Differentiation | Methods of | |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | **Developed** | **Conditions** | **Characterization** | **Reference** |
| Terato-carci-noma or teratoma | EC | Tumors containing tissue types from endoderm, mesoderm, and ectoderm | Bone morphogenetic protein-7<br>EC cells cultured without feeder cell layer<br>Transplantation of EC cells into mice | Morphology, histology, and cell staining<br>Observation of tissue types from endoderm, mesoderm, and ectoderm<br>Observation of extended self-renewal of EC cells<br>Analysis of chromosomes and specific genes<br>Detection of cell-specific proteins<br>Cytochemical assay | Andrews et al., 1984<br>Thompson et al., 1984<br>Pera, 1989 |

## Appendix D.v.
## Published Reports on Isolation and Differentiation of Human Adult Stem Cells

| Origin | | Cell Types | Differentiation | Methods of | |
|---|---|---|---|---|---|
| Tissue | Cell Type | Developed | Conditions | Characterization | Reference |
| Blood | Circulatory<br>Skeletal | Adipocyte<br>Osteocyte | Leukemia inhibitory factor (LIF)<br>Transplantation of stem cells into bg-nu-xid immunocompromised mice | Antibody labeling<br>Polymerase chain reaction | Kuznetsov, 2001 |
| Bone marrow | Angioblast (endothelial precursor) | Mature endothelia and newly formed blood vessels | Angioblasts isolated by mobilizing peripheral blood with granulocyte-colony stimulating factor<br>Angioblasts injected into rats with experimental myocardial infarction | Observation of neovascularization within myocardium from transplanted cells<br>Detection of improved cardiac function in experimental animals | Kocher et al., 2001 |
| | Hematopoietic stem cell (HSC) | Hepatocyte<br>Cholangiocyte | Bone marrow transplantation | Probed for presence and function of Y chromosome-containing liver cells<br>Measured expression of liver-specific proteins<br>Immunochemistry | Alison et al., 2000<br>Theise et al., 2000 |
| | Human marrow stromal | Stromal-derived cell engrafted in rat brain | Isolation of marrow stromal cell from human volunteers; injection of stromal cell into rat brain | Observation of engraftment, migration, and survival of stromal-derived cell in rat brain<br>Observation of loss of stromal cell functions<br>Antibodies to cell-surface proteins | Azizi et al., 1998 |
| | Mesenchymal stem cell<br>(MSC) | Adipocyte<br>Chondrocyte<br>Osteocyte | Fetal bovine serum, dexamethasone, isobutylxanthine, insulin, ascorbate, indomethacin, transforming growth factor-B3, and glycerol phosphate | Histology and immunofluorescence<br>Detection of lipids and specific enzyme activity of adipocytes and osteocytes<br>Specific staining for chondrocytes | Pittenger et al., 1999 |

***Appendix D.v.*** *(cont.)*

| Origin | | Cell Types | Differentiation | Methods of | |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | **Developed** | **Conditions** | **Characterization** | **Reference** |
| Bone marrow *(cont.)* | MSC | Neuron | Prolonged expansion of MSCs as undifferentiated cells<br>β-mercaptoethanol (BME)<br>Butylated hydroxyanisole (BHA) | Histology<br>Detection of numerous neuron-specific proteins via staining and antibody binding | Woodbury et al., 2000 |
| | MSC | Neuron | MSCs cultured with fetal rat brain cells<br>Epidermal growth factor<br>Brain-derived neurotrophic factor | Detection of nestin and nestin-gene expression<br>Detection of neuron-specific proteins | Sanchez-Ramos et al., 2000 |
| | MSC | Adipocyte<br>Bone marrow stromal cell<br>Cardiomyocyte<br>Chondrocyte<br>Myocyte<br>Thymic stromal cell | MSCs isolated from bone marrow<br>Transplantation of MSCs into fetal sheep | Analysis of human gene expression in sheep tissues<br>Confirmed presence of human cells by immunohistochemistry | Liechty et al., 2000 |
| Bone marrow<br>(fetal) | HSC | HSC<br>Red blood cell lineages<br>White blood cell lineages | Enrichment of hematopoietic cell populations by cell selection<br>Transplantation of bone marrow and thymus cells into mice | Establishment of long-term multilineage cultures of hematopoietic colonies<br>Fluorescence-activated cell sorting (FACS)<br>Engraftment of hematopoietic cells in mice | Baum et al., 1992 |
| Brain | Neural stem cell (NSC) | Muscle cell | Exposure of NSCs to myoblasts<br>Dissociation of NSC clusters<br>Transplantation of human NSCs into mice with induced muscle injury | Observation of differentiated skeletal muscle cells from primary and culture-derived NSCs<br>Demonstration of NSC engraftment in mice by detection of expression of specific genes | Galli et al., 2000 |

***Appendix D.v.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Brain (adult and neonatal) | Neural progenitor cell (NPC) | Astrocyte<br>Neuron<br>Oligodendrocyte | NPCs cultured in medium containing glutamine, amphotericin-B, antibiotics, fetal calf serum, basic fibroblast growth factor, epidermal growth factor, and platelet-derived growth factor AB<br>Transplantation of human central nervous system stem cells (hCNS-SCs) into mice | Observation of functional engraftment of NPCs into mouse brain<br>Antibody labeling of neuronal cell-surface proteins | Palmer et al., 2001 |
| Brain (fetal) | Human central nervous system stem cell (hCNS-SC) | Astrocyte<br>Neuron<br>Oligodendrocyte | Fibroblast growth factor-2, epidermal growth factor, lymphocyte inhibitory factor, neural survival factor-1, brain-derived and glial-derived neurotrophic factors | Observation of neurosphere formation and self-renewal of hCNS-SCs<br>Demonstration of engraftment, proliferation, migration, and neural differentiation of hCNS-SCs<br>FACS | Uchida et al., 2000 |
| Fat | Stromal vascular cell fraction of processed lipoaspirate | Adipocyte precursor<br>Osteocyte precursor<br>Chondrocyte precursor<br>Myocyte precursor | Co-cultured with mouse adipocytes, isobutylmethylxanthine, dexamethasone<br>Co-cultured with human osteoblasts, insulin, indomethacin, antibiotic/antimycotic dexamethasone, ascorbate, b-glycerophosphate, antibiotic/antimycotic<br>Co-cultured with human skeletal myocytes, insulin, transforming growth factor-B, ascorbate, antibiotic/antimycotic dexamethasone, hydrocortisone, antibiotic/antimycotic | Staining for lipid accumulation<br>Staining for alkaline phosphatase activity<br>Staining for bone formation<br>Staining for proteoglycan-rich matrix<br>Antibody binding to collagen II<br>Visualization of multinucleation<br>Staining for muscle protein: myosin<br>Antibody binding to MyoD1 | Zuk et al., 2001 |

***Appendix D.v.*** *(cont.)*

| Origin | | Cell Types Developed | Differentiation Conditions | Methods of Characterization | Reference |
|---|---|---|---|---|---|
| **Tissue** | **Cell Type** | | | | |
| Liver (fetal) | HSC | Hematopoietic progenitor cell (HPC)<br>Red blood cell lineages<br>White blood cell lineages | Co-culture of HSCs with mouse stromal cells<br>Implantation of fetal hematopoietic liver cells into immunocom-promised mice | Demonstration of differentiation into red and white blood cell lineages through colony-forming assays and detection of surface markers characteristic of the hematopoietic system | McCune et al., 1988<br>Namikawa et al., 1990 |
| Pancreas | Nestin-positive islet-derived progenitor cell (NIP) | Pancreatic<br>Hepatic | NIPs obtained from pancreatic islets and cultured for extended periods | Observation of extended proliferative, self-renewing, and multipotent capacity<br>Expression of hepatic and exocrine pancreatic markers<br>Demonstration of ductal and endocrine pancreatic features<br>Production of insulin and glucagons | Zulewski et al., 2000 |
| Umbilical cord blood | HPC | Most red and white blood cell lineages | Collection and sorting<br>Stimulation with colony-stimulating factors and interleukin-3 | Demonstration of multipotent progenitor, granulocyte-macrophage, and erythroid cell lines | Broxmeyer et al., 1989 |
| | HSC<br>Mesenchymal progenitor cell (MPC) | Most red and white blood cell lineages<br>Osteoblasts<br>Adipocytes | Mixtures of dexamethasone, ß-glycerol, ascorbate, insulin, isobutyl-methylxanthine, and indomethacin | Cell morphology<br>Cytochemical analysis of osteoblast and adipocyte products<br>Immunophenotyping | Erices et al., 1999 |

## Appendix D.vi.

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