3 ISOLATION OF STEM AND PRECURSOR CELLS FROM FETAL TISSUE pdf

1: Isolation of CD34+ Cells from Human Fetal Liver and Cord

Abstract. Generation of neurons, astrocytes, and oligodendrocytes in the nervous system involves a sequential process of differentiation. Initially, multipotent stem cells generate more restricted precursor cells, which go through additional stages of differentiation to generate fully differentiated progeny ().

The most-often discussed is their potential use in transplant therapyâ€”i. However, treatments for any of these diseases require that human ES cells be directed to differentiate into specific cell types prior to transplant. The research is occurring in several laboratories, but is limited because so few laboratories have access to human ES cells. Thus, at this stage, any therapies based on the use of human ES cells are still hypothetical and highly experimental [22 , 29 , 31] see Figure 3. Major Goals in the Development of Transplantation Therapies from Human ES Cell Lines Reproduced with permission from Stem Cells, One of the current advantages of using ES cells as compared to adult stem cells is that ES cells have an unlimited ability to proliferate in vitro, and are more likely to be able to generate a broad range of cell types through directed differentiation. Ultimately, it will also be necessary to both identify the optimal stage s of differentiation for transplant, and demonstrate that the transplanted ES-derived cells can survive, integrate, and function in the recipient. The potential disadvantages of the use of human ES cells for transplant therapy include the propensity of undifferentiated ES cells to induce the formation of tumors teratomas , which are typically benign. Because it is the undifferentiated cellsâ€”rather than their differentiated progenyâ€”that have been shown to induce teratomas, tumor formation might be avoided by devising methods for removing any undifferentiated ES cells prior to transplant. Also, it should be possible to devise a fail-safe mechanismâ€”i. Human ES derived cells would also be advantageous for transplantation purposes if they did not trigger immune rejection. The immunological status of human ES cells has not been studied in detail, and it is not known how immunogenic ES-derived cells might be. In general, the immunogenicity of a cell depends on its expression of Class I major histocompatability antigens MHC , which allow the body to distinguish its own cells from foreign tissue, and on the presence of cells that can bind to foreign antigens and "present" them to the immune system. The potential immunological rejection of human ES-derived cells might be avoided by genetically engineering the ES cells to express the MHC antigens of the transplant recipient, or by using nuclear transfer technology to generate ES cells that are genetically identical to the person who receives the transplant. The oocyte, thus "fertilized," could be cultured in vitro to the blastocyst stage. ES cells could subsequently be derived from its inner cell mass, and directed to differentiate into the desired cell type. The result would be differentiated or partly differentiated ES-derived cells that match exactly the immunological profile of the person who donated the somatic cell nucleus, and who is also the intended recipient of the transplantâ€”a labor intensive, but truly customized therapy [29]. For example, human ES cells could be used to study early events in human development. Still-unexplained events in early human development can result in congenital birth defects and placental abnormalities that lead to spontaneous abortion. By studying human ES cells in vitro, it may be possible to identify the genetic, molecular, and cellular events that lead to these problems and identify methods for preventing them [22 , 35 , 45]. Such cells could also be used to explore the effects of chromosomal abnormalities in early development. This might include the ability to monitor the development of early childhood tumors, many of which are embryonic in origin [32]. Human ES cells could also be used to test candidate therapeutic drugs. Currently, before candidate drugs are tested in human volunteers, they are subjected to a barrage of preclinical tests. These include drug screening in animal modelsâ€”in vitro tests using cells derived from mice or rats, for example, or in vivo tests that involve giving the drug to an animal to assess its safety. Although animal model testing is a mainstay of pharmaceutical research, it cannot always predict the effects that a candidate drug may have on human cells. For this reason, cultures of human cells are often employed in preclinical tests. These human cell lines have usually been maintained in vitro for long periods and as such often have different characteristics than do in vivo cells. These differences can make it

difficult to predict the action of a drug in vivo based on the response of human cell lines in vitro. Human ES cells could be employed to screen potential toxins. The reasons for using human ES cells to screen potential toxins closely resemble those for using human ES-derived cells to test drugs above. Toxins often have different effects on different animal species, which makes it critical to have the best possible in vitro models for evaluating their effects on human cells. Finally, human ES cells could be used to develop new methods for genetic engineering see Figure 3. Currently, the genetic complement of mouse ES cells in vitro can be modified easily by techniques such as homologous recombination. This is a method for replacing or adding genes, which requires that a DNA molecule be artificially introduced into the genome and then expressed. Using this method, genes to direct differentiation to a specific cell type or genes that express a desired protein product might be introduced into the ES cell line. Ultimately, if such techniques could be developed using human ES cells, it may be possible to devise better methods for gene therapy [35] see Chapter Assessing Human Stem Cell Safety. Since , research teams have refined the techniques for growing human ES cells in vitro [1 , 20 , 38]. Collectively, the studies indicate that it is now possible to grow human ES cells for more than a year in serum-free medium on feeder layers. The cells have normal karyotype and are pluripotent; they generate teratomas that contain differentiated cell types derived from all three primary germ layers. The long-term cultures of human ES cells have active telomerase and maintain relatively long telomeres, another marker of proliferating cells. Overall, the pluripotent cells that can be generated in vitro from human ES cells and human EG cells are apparently not equivalent in their potential to proliferate or differentiate. ES cells are derived from the inner cell mass of the preimplantation blastocyst, approximately 5 days post-fertilization, whereas human EG cells are derived from fetal primordial germ cells, 5 to 10 weeks post-fertilization. ES cells can proliferate for up to population doublings, while cells derived from embryoid bodies that are generated from embryonic germ cells fetal tissue double a maximum of 70 to 80 times in vitro. ES cells appear to have a broader ability to differentiate. Both kinds of cells spontaneously generate neural precursor-type cells widely regarded as a default pathway for differentiation , and both generate cells that resemble cardiac myocytes [19 , 45]. However, human ES and EG cells in vitro will spontaneously generate embryoid bodies that consist of cell types from all three primary germ layers [1 , 20 , 38 , 42]. Scientists are just beginning to understand the biology of human embryonic stem cells, and many key questions remain unanswered or only partly answered. For example, in order to refine and improve ES cell culture systems, it is important that scientists identify the mechanisms that allow human ES cells in vitro to proliferate without differentiating [29]. Once the mechanisms that regulate human ES proliferation are known, it will likely be possible to apply this knowledge to the long-standing challenge of improving the in vitro self-renewal capabilities of adult stem cells. It will also be important to determine whether the genetic imprinting status of human ES cells plays any significant role in maintaining the cells, directing their differentiation, or determining their suitability for transplant. One of the effects of growing mouse blastocysts in culture is a change in the methylation of specific genes that control embryonic growth and development [23]. Do similar changes in gene imprinting patterns occur in human ES cells or blastocysts? If so, what is their effect on in vitro development and on any differentiated cell types that may be derived from cultured ES cells? Efforts will need to be made to determine whether cultures of human ES cells that appear to be homogeneous and undifferentiated are, in fact, homogeneous and undifferentiated. Is it possible that human ES cells in vitro cycle in and out of partially differentiated states? And if that occurs, how will it affect attempts to direct their differentiation or maintain the cells in a proliferating state [28]? Scientists will need to identify which signal transduction pathways must be activated to induce human ES cell differentiation along a particular pathway. This includes understanding ligand-receptor interaction and the intracellular components of the signaling system, as well as identifying the genes that are activated or inactivated during differentiation of specific cell types [29]. Identifying intermediate stages of human ES cell differentiation will also be important. As human ES cells differentiate in vitro, do they form distinct precursor or progenitor cells that can be identified and isolated? If ES cells do form such intermediate cell types, can the latter be maintained and expanded? Would such precursor or progenitor

cells be useful for therapeutic transplantation [19]? Finally, scientists will need to determine what differentiation stages of human ES-derived cells are optimal for other practical applications. For example, what differentiation stages of ES-derived cells would be best for screening drugs or toxins, or for delivering potentially therapeutic drugs? Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera Differentiation in vivo and in vitro. Teratocarcinomas and human embryology: Blastocyst transfer in human in vitro: Isolation and culture of inner cell mass cells from human blastocysts. Co-culture techniques for blastocyst transfer and embryonic stem cell production. Behaviour of human embryos in vitro in the first 14 days: The benefits to human IVF by transferring embryos after the in vitro embryonic block: Handbook on blastocyst culture, Singapore: Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Zona hardening, zona drilling and assisted hatching: Establishment in culture of pluripotential cells from mouse embryos. Blastocyst transfer after enzymatic treatment of the zona pellucida: Mouse genital ridges in organ culture: Culture and transfer of human blastocysts. Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers. Factors affecting the success of human blastocyst development and pregnancy following in vitro fertilization and embryo transfer. Human embryonic stem cell technology. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Multipotentiality of single embryonal carcinoma cells. Teratocarcinomas and mammalian embryogenesis. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Effect of steel factor and leukaemia inhibitory factor on murine primordial germ cells in culture. Embryonic stem cells for medicine. Isolation and characterization of a multipotentclone of human embryonal carcinoma cells. Human embryonic stem cells. Properties and uses of embryonic stem cells: Long-term proliferation of mouse primordial germ cells in culture. Reubinoff BE, Pera, M. Embryonic stem cell lines from human blastocysts: Ultrastructure of the human egg. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Derivation of pluripotent stem cells from cultured human primordial germ cells.

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2: Neural Stem Cells

The following protocol, outlined in Figure 1, provides our current method for the isolation of human fetal pancreatic cells, called ICCs from whole human fetal pancreas and imaging of these cells. This protocol requires an initial preparation of cells from tissue, which can be subsequently grown as a monolayer or in suspension.

Neurons transmit information through action potentials and neurotransmitters to other neurons, muscle cells or gland cells. Astrocytes and oligodendrocytes, collectively called glial cells, play important roles of their own, in addition to providing a critical support role for optimal neuronal functioning and survival. During mammalian embryogenesis, CNS development begins with the induction of the neuroectoderm, which forms the neural plate and then folds to give rise to the neural tube. Within these neural structures there exists a complex and heterogeneous population of neuroepithelial progenitor cells NEPs , the earliest neural stem cell type to form. In the later stage of neural development, NSCs switch to asymmetric division cycles and give rise to lineage-restricted progenitors. Intermediate neuronal progenitor cells are formed first, and these subsequently differentiate to generate to neurons. Following this neurogenic phase, NSCs undergo asymmetric divisions to produce glial-restricted progenitors, which generate astrocytes and oligodendrocytes. The later stage of CNS development involves a period of axonal pruning and neuronal apoptosis, which fine tunes the circuitry of the CNS. A previously long-held dogma maintained that neurogenesis in the adult mammalian CNS was complete, rendering it incapable of mitotic divisions to generate new neurons, and therefore lacking in the ability to repair damaged tissue caused by diseases e. However, there is now strong evidence that multipotent NSCs do exist, albeit only in specialized microenvironments, in the mature mammalian CNS. This discovery has fuelled a new era of research into understanding the tremendous potential that these cells hold for treatment of CNS diseases and injuries. Identification of Neural Stem Cells Neurobiologists routinely use various terms interchangeably to describe undifferentiated cells of the CNS. The inappropriate use of these terms to identify undifferentiated cells in the CNS has led to confusion and misunderstandings in the field of NSC and neural progenitor cell research. However, these different types of undifferentiated cells in the CNS technically possess different characteristics and fates. For clarity, the terminology used here is: Multipotent cells which are able to self-renew and proliferate without limit, to produce progeny cells which terminally differentiate into neurons, astrocytes and oligodendrocytes. The non-stem cell progeny of NSCs are referred to as neural progenitor cells. Neural progenitor cells have the capacity to proliferate and differentiate into more than one cell type. Neural progenitor cells can therefore be unipotent, bipotent or multipotent. A distinguishing feature of a neural progenitor cell is that, unlike a stem cell, it has a limited proliferative ability and does not exhibit self-renewal. As used here, this refers to a mixed population of cells consisting of all undifferentiated progeny of neural stem cells, therefore including both neural progenitor cells and neural stem cells. The term neural precursor cells is commonly used to collectively describe the mixed population of NSCs and neural progenitor cells derived from embryonic stem cells and induced pluripotent stem cells. Prior to , numerous reports demonstrated evidence of neurogenesis and limited in vitro proliferation of neural progenitor cells isolated from embryonic tissue in the presence of growth factors. Notable exceptions included several studies in the s that clearly identified a region of the adult brain that exhibited proliferation the forebrain subependyma 6 but this was believed to be species-specific and was not thought to exist in all mammals. In the early s, cells that responded to specific growth factors and exhibited stem cell features in vitro were isolated from the embryonic and adult CNS. The location of stem cells in the adult brain was later identified to be within the striatum,9 and researchers began to show that cells isolated from this region, and the dorsolateral region of the lateral ventricle of the adult brain, were capable of differentiating into both neurons and glia. During embryogenesis, neural precursor cells are derived from the neuroectoderm and can first be detected during neural plate and neural tube formation. As the embryo develops, neural stem cells can be identified in nearly all regions of the embryonic mouse, rat and human CNS, including the septum, cortex, thalamus, ventral

mesencephalon and spinal cord. NSCs isolated from these regions have a distinct spatial identity and differentiation potential. NPCs in this niche are relatively quiescent under normal physiological conditions, but can be induced to proliferate and to repopulate the SVZ following irradiation. TAPs and neuroblasts migrate through the rostral migratory stream RMS and further differentiate into new interneurons in the olfactory bulb. This ongoing neurogenesis, which is supported by the NSCs in the SVZ, is essential for maintenance of the olfactory system, providing a source of new neurons for the olfactory bulb of rodents and the association cortex of non-human primates. Lineage tracing studies have mapped the neural progenitor cells to the dorsal region of the hippocampus, in a collapsed ventricle within the dentate gyrus. Whereas the SVZ NSCs play a maintenance role, it is thought that hippocampal neurogenesis serves to increase the number of new neurons and contributes to hippocampal growth throughout adult life. Neural Stem Cell Culture Systems In vitro methodologies designed to isolate, expand and functionally characterize NSC populations have revolutionized our understanding of neural stem cell biology, and increased our knowledge of the genetic and epigenetic regulation of NSCs. Neural induction and differentiation of pluripotent stem cells: Early NPCs can be derived from mouse and human PSCs, which include embryonic stem cells ESCs and induced pluripotent stem cells iPSCs , using appropriate neural induction conditions at the first stage of differentiation. The NPCs present in the neural rosette structures are then isolated, and can be propagated to allow NPC expansion, while maintaining the potential to generate neurons and glial cells. More recently, studies have shown that neural induction of PSCs can also be achieved in a monolayer culture system, wherein human ESCs and iPSCs are plated onto a defined matrix, and exposed to inductive factors. Cerebral organoids recapitulate features of human brain development, including the formation of discrete brain regions featuring characteristic laminar cellular organization. The neurosphere culture system has been widely used since its development as a method to identify NSCs. In the neurosphere culture system, NSCs, as well as neural progenitor cells, begin to proliferate in response to these mitogens, forming small clusters of cells after 2 - 3 days. The clusters continue to grow in size, and by day 3 - 5, the majority of clusters detach from the culture surface and begin to grow in suspension. At this point, the neurospheres should be passaged to prevent the cell clusters from growing too large, which can lead to necrosis as a result of a lack of oxygen and nutrient exchange at the neurosphere center. To passage the cultures, neurospheres are individually, or as a population, mechanically or enzymatically dissociated into a single cell suspension and replated under the same conditions as the primary culture. NSCs and neural progenitor cells again begin to proliferate to form new cell clusters that are ready to be passaged approximately 5 - 7 days later. By repeating the above procedures for multiple passages, NSCs present in the culture will self-renew and produce a large number of progeny, resulting in a relatively consistent increase in total cell number over time. Neurospheres derived from embryonic mouse CNS tissue treated in this manner can be passaged for up to 10 weeks with no loss in their proliferative ability, resulting in a greater than fold increase in total cell number. NSCs and neural progenitors can be induced to differentiate by removing the mitogens and plating either intact neurospheres or dissociated cells on an adhesive substrate, in the presence of a low serum-containing medium. After several days, virtually all of the NSCs and progeny will differentiate into the three main neural cell types found in the CNS: While the culture medium, growth factor requirements and culture protocols may vary, the neurosphere culture system has been successfully used to isolate NSCs and progenitors from different regions of the embryonic and adult CNS of many species including mouse, rat and human. When plated under these conditions, the neural stem and progenitor cells will attach to the substrate-coated cultureware, as opposed to each other, forming an adherent monolayer of cells, instead of neurospheres. To passage the cultures, cells are detached from the surface by enzymatic treatment and replated under the same conditions as the primary culture. It has been reported that NSCs cultured under adherent monolayer conditions undergo symmetric divisions in long-term culture. Several studies have suggested that culturing CNS cells in neurosphere cultures does not efficiently maintain NSCs and produces a heterogeneous cell population, whereas culturing cells under serum-free adherent culture conditions does maintain NSCs. It is important that in vitro methodologies for NSC research are designed with this caveat in

mind, and with a clear understanding of what the methodologies are purported to measure. Similar to stem cells in other systems, the phenotype of CNS stem cells has not been completely determined. Expression, or lack of expression, of CD34, CD and CD45 antigens has been used as a strategy for the preliminary characterization of potential CNS stem cell subsets. However, none of these markers are uniquely expressed by NSCs; many are also expressed by neural progenitor cells and other nonneural cell types. Studies have demonstrated that stem cells in a variety of tissues, including bone marrow, skeletal muscle and fetal liver can be identified by their ability to efflux fluorescent dyes such as Hoechst ALDH-bright cells from embryonic rat and mouse CNS have been isolated and shown to have the ability to generate neurospheres, neurons, astrocytes and oligodendrocytes in vitro, as well as neurons in vivo, when transplanted into the adult mouse cerebral cortex. They also initiate tumors that phenocopy the parent tumor in immunocompromised mice. However, there is some indication that these mitogens are not required when culturing BTSCs. Contrary to the beliefs of the past century, the adult mammalian brain retains a small number of true NSCs located in specific CNS regions. The identification of CNS-resident NSCs and the discovery that adult somatic cells from mouse and human can be reprogrammed to a pluripotent state, and then directed to differentiate into neural cell types, has opened the door to new therapeutic avenues aimed at replacing lost or damaged CNS cells. This may include transplantation of neural progenitors derived from fetal or adult CNS tissue, or pluripotent stem cells. Recent research has shown that adult somatic cells can be directly reprogrammed to specific cell fates, such as neurons, using appropriate transcriptional factors, bypassing the need for an induced pluripotent stem cell intermediate. Together, these reagents help to advance neuroscience research and assist in its transition from the experimental to the therapeutic phase. Annu Rev Neurosci Altman J, et al. Cell Stem Cell 6: Mol Cell Neurosci Cereb Cortex 16 Suppl 1: Cell Stem Cell 4: Cell Stem Cell 5: Hum Mol Genet Nat Rev Cancer 6: Cell Death Differ Int J Cancer Pediatr Blood Cancer PloS One 6 1: Cell Stem Cell 1:

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3: Isolation, Culture, and Imaging of Human Fetal Pancreatic Cell Clusters

Isolation of cardiovascular precursors from the fetal heart. Human fetal ventricles were isolated, minced, digested, and processed into primary cultures (Fig. 1A).The cell population that adhered in less than 24 h represents the nonmyocyte population containing CPCs (Fig. 1B).

This item can be ordered from any standard company. C CO2 Incubator, catalog number: Collect fresh human umbilical cord after full-term birth normal or cesarean with informed consent Figure 1 and transport it to the lab in an empty sterile 50 ml centrifuge tube on ice. Fresh umbilical cord after collection Rinse off the blood and blood clots using normal saline. This incubation step in saline containing antibiotic-antimycotic is carried out mainly to disinfect the umbilical cord tissue. The umbilical cord cut into cm long pieces After 2 h, inside the laminar flow tissue culture hood, rinse the cord pieces three times with sterile PBS Video 1. Wash thoroughly three more times with PBS to remove any traces of ethanol Video 1. Transfer one cord piece into an empty 10 cm cell culture dish, while the tube containing the other pieces can be stored on ice. Using forceps try to straighten the twists, if any, in the cord piece and using a scalpel fixed to a scalpel holder make a longitudinal slit along the length Video 2. Removal of artery from umbilical cord piece There are two arteries and one vein inside the cord. Cut open flaps of tissue to expose an artery and then trace the artery using scissors and forceps and remove it. Chop the excised tissue into smaller mm pieces with the help of the scalpel and place in an empty 35 mm tissue culture dish using a fine forceps Video 3. Plating small explants of WJ portion of human umbilical cord Allow the cord pieces to attach for about 5 min with air drying. Next add 2 ml of warm fresh MSC isolation medium carefully, drop-wise and gently, taking care not to dislodge the tissue pieces Video 3. Place about explants in each 35 mm tissue culture dish and use about four to five 35 mm tissue culture dishes per umbilical cord sample. Discard the remaining tissue pieces in a biohazard waste bag. All the liquid waste should be collected in a beaker containing sodium hypochlorite solution and discarded appropriately. After 48 h, give the first medium change with 2 ml of warm fresh MSC isolation medium. Following this, media changes are given with 2 ml of fresh MSC isolation medium every 72 h. After about days, once enough cells have come out from the explants Figure 3 , remove the cord pieces using 1 ml blunt-end tips and add 2 ml of warm fresh MSC isolation medium. Phase contrast image of MSCs emerging from an umbilical cord tissue piece explant Establishment of MSC culture After h of cord piece removal and depending on confluency, carefully aspirate medium and wash cells twice with DPBS. Trypsin can be used as a substitute for TrypLE. Add 1 ml of warm fresh MSC growth medium to the cells and transfer them to a 15 ml centrifuge tube and centrifuge at room temperature for 2 min at x g. Mix a small aliquot of cell suspension with an equal volume of trypan blue, and count the live non-blue cells using a hemocytometer. No medium change required in between. Add 1 ml of warm fresh MSC growth medium to the cells and transfer them to a 15 ml centrifuge tube. Take an aliquot for counting and centrifuge the remaining cells at x g for 2 min at room temperature. FrostyTM freezing container containing isopropanol. Once the cell suspension has thawed, inside the laminar flow hood add 1 ml of warm fresh MSC growth medium to the cell suspension in the cryogenic vial and transfer the contents to a 15 ml centrifuge tube containing ml of additional warm fresh MSC growth medium. Centrifuge the cells at room temperature at x g for min to wash off the DMSO. Aspirate the supernatant and resuspend the cell pellet in 0. Mix well by pipetting, count the live cells and plate up to x cells on a 35 mm tissue culture plate in 2 ml of warm fresh MSC growth medium. During thawing and revival of MSCs, cells are plated at a higher density in order to maximize recovery. Transfer the dish to a CO2 incubator and replace medium with 2 ml of warm fresh MSC growth medium the next day. After 48 h of reviving and plating, detach cells with TrypLE and seed for the next passage. Wash the harvested cells times with ice cold PBS by centrifuging at x g for 2 min. Mouse isotype antibodies can serve as controls Figure 4. Open histograms represent background signal while shaded histograms indicate positive reactivity with the indicated antibodies. Data analysis WJ-MSCs, isolated from human umbilical cord, are analysed for surface marker

expression profile at passage All analyses are standardized against control cells incubated with PE-conjugated mouse IgG isotype antibody. Notes For MSC isolations, cords are usually collected from healthy donors. After collection, umbilical cords should be transported to the laboratory and processed as soon as possible, preferably within one hour. All plasticwares like microcentrifuge tubes and pipet tips, and surgical instruments were sterilized by autoclaving before use in cell culture.

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4: Isolation and Establishment of Mesenchymal Stem Cells from

Stem Cells, Neuroblasts, and Glioblasts from Fetal Tissue 31 c. Coat the dish with 1 mL ï¬• bronectin solution at 4Â°C overnight.

To view a copy of this license, visit http: Abstract For almost 30 years, scientists have demonstrated that human fetal ICCs transplanted under the kidney capsule of nude mice matured into functioning endocrine cells, as evidenced by a significant increase in circulating human C-peptide following glucose stimulation However in vitro, genesis of insulin producing cells from human fetal ICCs is low10; results reminiscent of recent experiments performed with human embryonic stem cells hESC , a renewable source of cells that hold great promise as a potential therapeutic treatment for type 1 diabetes. Like ICCs, transplantation of partially differentiated hESC generate glucose responsive, insulin producing cells, but in vitro genesis of insulin producing cells from hESC is much less robust A complete understanding of the factors that influence the growth and differentiation of endocrine precursor cells will likely require data generated from both ICCs and hESC. While a number of protocols exist to generate insulin producing cells from hESC in vitro, far fewer exist for ICCs10,23, Part of that discrepancy likely comes from the difficulty of working with human fetal pancreas. Towards that end, we have continued to build upon existing methods to isolate fetal islets from human pancreases with gestational ages ranging from 12 to 23 weeks, grow the cells as a monolayer or in suspension, and image for cell proliferation, pancreatic markers and human hormones including glucagon and C-peptide. ICCs generated by the protocol described below result in C-peptide release after transplantation under the kidney capsule of nude mice that are similar to C-peptide levels obtained by transplantation of fresh tissue6. Medicine, Issue 87, human fetal pancreas, islet cell cluster ICC , transplantation, immunofluorescence, endocrine cell proliferation, differentiation, C-peptide Download video file. The ability to regulate proliferation and differentiation of human pancreatic precursor cells into insulin-producing cells that meet the metabolic demands of an insulin-deficient state remains a critical building block for a cell-based therapy to treat type 1 diabetes. Until the advent of hESC derived insulin-producing cells, human fetal pancreatic endocrine cells or their precursors were viewed as potential sources of cells for clinical transplantation. Although the scientific and regulatory landscape has changed in the past few years, there remains a vital need to understand how the human fetal pancreas develops. Many now view therapeutic use of human fetal cells as unlikely, however if effective and safe methods to expand the cells were established, therapeutic use of these cells could again be explored. A major hurdle that remains is that in vitro transformation of human fetal pancreatic cells aggregates ICCs into glucose responsive, insulin secreting endocrine cells is currently an inefficient process. Although much work over almost 30 years has elucidated and delineated the expression profile of transcription factors required for the development of endocrine pancreas, there remain gaps in our knowledge about how temporal expression of transcription factors is regulated and related to cell function. Recently, the stem cell field has employed the accumulated knowledge about temporal transcription factor expression during islet development to drive the production of cells that express the markers of mature endocrine cells. Although genesis of insulin producing cells from hESC and induced pluripotent cells iPSC has made substantial and significant advances in the past few years, the most effective protocols require two distinct differentiation phases: To move forward, advances in understanding the biology underlying islet maturation in vivo, regardless of cell source, must be understood at a biochemical level. The similarity between the results obtained with ICCs and hESC suggests that a number of critical biochemical processes that regulate the transition of human pancreatic precursor cells into mature, glucose responsive, insulin secreting cells in vitro remain unknown. A central part of this understanding will be to develop novel methodologies to derive functional endocrine cell populations from pancreatic progenitor cells and hESC will require not only biochemical approaches that elucidate the maturation events, but methods to analyze the changes. Why do we believe that imaging human fetal pancreatic cells is a critical aspect of identifying

changes in islet maturation? The answer lies partially in the historical quest to generate insulin producing cells. A limitation to the exploration of human fetal pancreas development is that the gestational ages that can be legally used only give rise to heterogeneous cell aggregates. However, after transplantation and in vivo maturation, a large majority of cells express endocrine markers 6, The results from these studies are being echoed today with hESC differentiation protocols that are only able to generate modest populations of single hormone positive endocrine cells after in vitro differentiation. In vitro methods to enhance populations of hormone positive cells will likely provide insight into in vivo islet maturation. The similarities between human fetal pancreatic cell and hESC maturation can be extended to cell function. However, upon transplantation into nude mice and maturation, both human islet-like clusters and insulin producing cells derived from hESC exhibit an endocrine phenotype. These results indicate that transplantation provides context and unidentified cues that promote islet maturation. Optimized imaging protocols, such as the one described here, for human fetal pancreatic cells will aid in the search to identify factors that modulate and accelerate maturation. Fundamental biochemical exploration of human fetal pancreatic cells and hESC differentiated towards an endocrine lineage at this stage of development is essential for measuring efficiency of maturation. The following protocol, outlined in Figure 1, provides our current method for the isolation of human fetal pancreatic cells, called ICCs from whole human fetal pancreas and imaging of these cells. This protocol requires an initial preparation of cells from tissue, which can be subsequently grown as a monolayer or in suspension. Preparation of cells for imaging of commonly used markers of endocrine cell proliferation and maturation is described. Generation and imaging of ICCs in the absence or presence of a variety of chemical modifying agents provides a rapid method, compared with transplantation models, to help identify culture conditions and compounds that accelerate maturation of human fetal pancreatic cells into fully functional exocrine, ductal, or hormone producing pancreatic cells. Protocol Notes before getting started: Informed consent for tissue donation, storage, and use of the samples was obtained from the donors by the center. It is essential to maintain both the human fetal pancreas and the container holding the pancreas on ice during the entire procedure. The dissociation and digestion should be performed as fast as possible. Send the clinic where the human fetal pancreas was obtained from the buffer for storage and transport of the pancreas. Filter sterilize the solution with a 0. In a tissue culture hood, set out 2 sterile Petri dishes, a sterile small crucible if a crucible is unavailable, a small beaker may be substituted , sterilized scissors, and forceps. Aspirate liquid from the tube containing the fetal pancreas, leaving approximately 1 ml. For these experiments, fetal ICCs are generated from fresh pancreata with gestational ages ranging from 9 to 23 weeks. Isolation from pancreata at earlier gestional ages is difficult due to the size of the pancreas. The protocol is likely applicable to gestational ages beyond 23 weeks, however acquisition of pancreata after this time is difficult because of federal U. Occasionally, the fetal pancreas arrives with splenic, fat, or connective tissue attached from the original dissection. The extra tissue is easily visible to the naked eye and should be removed from the pancreas before starting the protocol. Move the cleaned pancreas to the small sterile crucible using sterile forceps and place in an ice bucket. Place the crucible in a holder for a 50 ml centrifuge tube and, using 2 pairs of scissors, vigorously slice the pancreas for several minutes. Usually min, but the time depends on the size and firmness of the tissue. Cutting technique is important. Hold one side of the scissors in a fixed position moving only the opposite handles. The tips of the scissors should rest on the bottom of the crucible. Continue with rapid scissor motion to break the pancreas into small pieces. The smaller the pieces of tissue, the better the collagenase will work. Do not check more frequently than once during the first 5 min. Digestion time depends on the tissue quality, as little as 6 min may be sufficient. The end point is when the particles are small and uniform. Fill the vial ml with cold HBSS to stop the collagenase activity. Place on ice and allow the clumps to settle for 10 min. Often at this point, some cell death has occurred and DNA is released into the media. After the tissue in the scintillation vial has settled for 10 min, aspirate off the top layer leaving it slightly less than half full ml. Transfer the cells to a 15 ml conical tube, add 5 ml HBSS. Centrifuge for 5 min at x g. Plate on 60 mm Petri dish. Cells should be left in suspension for 72 hr to aggregate and form ICCs. After 72 hr in suspension, cells

can be plated on the matrix of the human cell line HTB9 matrix as described previously Regardless of growth conditions, the media should be changed every 48 hr. BrdU reagent is diluted 1: For cells in suspension: Centrifuge for 5 min at x g and aspirate culture medium. If the ICCs are going to be embedded in paraffin, please follow optional protocol 3. Remove culture medium and wash cells with PBS as described above. Incubate the cells with 0. Wash the cells with Working Buffer Blocking Buffer diluted 1: Dilute the antibody in working buffer. To stain multiple epitopes, a cocktail of antibodies may be applied. As a control, use IgG or pre immune serum in place of the specific antibody. Control samples must be incubated with control IgG from the same species as the primary antibody being used. Incubate the primary antibody either 1. Dilute the secondary antibody at 1: It is important to note that all secondary antibodies are light sensitive. Cover the samples in aluminum foil to prevent loss of signal. Incubate for 1 hr at RT. To visualize the nuclei, DAPI can be added at 1: This is useful for quantitation of protein expression in the total cell population. For cells grown on a coverslip, gently lift the coverslip with forceps while immersed in the buffer; wash it by immersing in PBS. For cells grown in a 6-well plate, add PBS to wash and aspirate. At this point they are ready to examine under an inverted microscope. Touch the edge of the coverslip gently on a Kimwipe to get rid of excess PBS. Add a drop of mounting gel on a glass slide and invert the coverslip onto the slide. Remove excess mounting gel by aspiration. Transfer the ICCs incubated in suspension to a 15 ml conical tube, and centrifuge at 1, x g for 5 min at room temperature. Do not mix the pellet, unless the pellet is very large. Mix by gently flicking the tube, but do not form bubbles. Use a 21 G needle to dislodge the pellet and place in a fresh 15 ml conical tube. Paraffin embedding and the preparation of sections on slides can be done on site using a microtome or by your core microscopy facilities.

3 ISOLATION OF STEM AND PRECURSOR CELLS FROM FETAL TISSUE pdf

5: NaÃ¯ve Adult Stem Cells Isolation from Primary Human Fibroblast Cultures | Protocol

5 Isolation of Neural Stem and Precursor Cells from Rodent Tissue Yuanyuan Wu, Ying Liu, Jonathan D. Chesnut, and Mahendra S. Rao Summary Isolation and characterization of neural stem cells and lineage-specific progenitors.

The invention also relates to methods for collecting, isolating, processing and cryopreserving of the fetal stem and progenitor cells of the invention. Importantly, the invention meets an urgent need in regenerative medicine while at the same time ensures utility of the product compositions because it utilizes fetal materials from second trimester weeks of gestation spontaneously lost pregnancies which are free of moral or ethical burdens. Importantly, the invention meets an urgent need while at the same time ensures utility of the product compositions because it utilizes fetal materials from second trimester weeks of gestation spontaneously lost pregnancies which are free of moral or ethical burdens. More particularly, the present invention relates to hematopoietic reconstitution and replacement in patients with various diseases and metabolic disorders. Regenerative medicine is a newly evolving method of targeted treatments of a large number of life threatening diseases. It involves three therapeutic technologiesâ€”cellular therapy, cell engineering and gene therapyâ€”all three involved in the utilization of human tissues. Cellular therapy has the broadest therapeutic applications in various diseases such as immunodeficiencies, hemoglobinopathies, metabolic disorders, diseases of the nervous system and some malignancies. Cell engineering is another promising area of novel therapy that may have a major impact on management of cardiac ischemic diseases, treatment of bone and joint diseases, and chronic wounds with bone marrow or mesenchymal stem cells. Gene therapy is another form of technology that has held considerable promise although unexpected long term effects of gene therapy, such as malignancies and death have limited its clinical application. Cellular therapies as well as cell engineering, the most important technologies in regenerative medicine, require a large supply of stem cells. At present there is an insufficient supply of human stem cells for current applications and this lack will be compounded as the regenerative medicine reaches its full potential. The current sources of human hematopoietic stem cells include adult peripheral blood, cord blood, and the adult bone marrow. The morphologically recognizable and functionally capable cells circulating in blood include erythrocytes, neutrophilic, eosinophilic, and basophilic granulocytes, B-, T-, nonB-, non T-lymphocytes, and platelets. These mature cells derive from and are replaced, on demand, by morphologically recognizable dividing precursor cells for the respective lineages such as erythroblasts for the erythrocyte series, myeloblasts, promyelocytes and myelocytes for the granulocyte series, and megakaryocytes for the platelets. The precursor cells derive from more primitive cells that can simplistically be divided into two major subgroups: The definitions of stem and progenitor cells are operational and depend on functional, rather than on morphological, criteria. Stem cells have extensive self-renewal or self-maintenance capacity, a necessity since absence or depletion of these cells could result in the complete depletion of one or more cell lineages, events that would lead within a short time to disease and death. Some of the stem cells differentiate upon need, but some stem cells or their daughter cells produce other stem cells to maintain the precious pool of these cells. Thus, in addition to maintaining their own kind, pluripotent stem cells are capable of differentiating into several sublines of progenitor cells with more limited or no self-renewal capacity or no self-renewal capacity. These progenitor cells ultimately give rise to the morphologically recognizable precursor cells. The progenitor cells are capable of proliferating and differentiating along one, or more than one, of the myeloid differentiation pathways. Under the appropriate growth conditions, the stem or progenitor cells will go through a sequence of proliferation and differentiation yielding mature end stage progeny, which thus allows the determination of the cell type giving rise to the colony. If the colony contains granulocytes, macrophages, erythrocytes, and megakaryocytes the precursors to platelets , then the cells giving rise to them would have been pluripotent cells. To determine if these cells have self-renewal capacities, or stemness, and can thus produce more of their own kind, cells from these colonies can be replated in vivo or in vitro. Those colonies, which upon replating into secondary culture plates, give

rise to more colonies containing cells of multilineages, would have contained cells with some degree of stemness. The stem cell and progenitor cell compartments are themselves heterogeneous with varying degrees of self-renewal or proliferative capacities. Self-renewal would appear to be greater in those stem cells with the shortest history of cell division, and this self-renewal would become progressively more limited with subsequent division of the cells. Reconstitution of the hematopoietic system has been accomplished by bone marrow transplantation. The stem and progenitor cells in donated bone marrow can multiply and replace the blood cells responsible for protective immunity, tissue repair, clotting, and other functions of the blood. In successful bone marrow transplantation, the blood, bone marrow, spleen, thymus and other organs of immunity are repopulated with cells derived from the donor. Cryopreservation of Cellsâ€"Freezing and thawing are destructive to most living cells. Upon cooling, as the external medium freezes, cells equilibrate by losing water, thus increasing intracellular solute concentration. Both intracellular freezing and solution effects are responsible for cell injury. It has been proposed that freezing destruction from extracellular ice is essentially a plasma membrane injury resulting from osmotic dehydration of the cell. Cryoprotective agents and optimal cooling rates can protect against cell injury. Cryoprotection by solute addition is thought to occur by two potential mechanisms: Different optimal cooling rates have been described for different cells. Various groups have looked at the effect of cooling velocity or cryopreservatives upon the survival or transplantation efficiency of frozen bone marrow cells or red blood cells. The successful recovery of human bone marrow cells after long-term storage in liquid nitrogen has been described , American Type Culture Collection, Quarterly Newsletter 3 4: There are many methods of cryopreservation and many cryoprotective cocktails have been proposed. Experience with standard protocols based on those designed for cord blood stem cells have clearly indicated that there is need for further improvement in the procedures for cryopreservation. Consequently the present invention fucusses effort in this area on minimizing the toxic effects of DMSO by radically decreasing its concentration in the freezing cocktail. Gene Therapy refers to the transfer and stable insertion of new genetic information into cells for the therapeutic treatment of diseases or disorders. The foreign gene is transferred into a cell that proliferates to spread the new gene throughout the cell population. Thus stem cells, or pluripotent progenitor cells, are usually the target of gene transfer, since they are proliferative cells that produce various progeny lineages which will potentially express the foreign gene. Most studies in gene therapy have focused on the use of hematopoietic stem cells. High efficiency gene transfer systems for hematopoietic progenitor cell transformation have been investigated for use. Reports on the development of viral vector systems indicate a higher efficiency of transformation than DNA-mediated gene transfer procedures e. Recombinant retrovirus vectors have been widely used experimentally to transduce hematopoietic stem and progenitor cells. Genes that have been successfully expressed in mice after transfer by retrovirus vectors include human hypoxanthine phosphoribosyl transferase. Bacterial genes have also been transferred into mammalian cells, in the form of bacterial drug resistance gene transfers in experimental models. Introduction of drug resistance genes into hematopoietic stem cells has been accomplished using a retroviral vector system. Adenovirus vectors have been used successfully to transduce mammalian cell lines to neomycin resistance. Other viral vector systems that have been investigated for use in gene transfer include parvoviruses and vaccinia viruses. Other methods of gene transfer including microinjection, electroporation, liposomes, chromosome transfer, and transfection techniques have been published in literature and are incorporated herein. However, there are still very significant problems to overcome before embryonic stem cells can be utilized in therapy. Likewise the use of fetal stem cells derived from elective abortions is plagued with many problems. Most elective abortions occur early in gestation hence there are little or no hematopoeitic cells in long bones because they have still not translocated to their ultimate destination from the liver. Moreover, there are significant moral and ethical problems in using fetal tissue derived from elective abortions. The present invention has overcome some of these problems by establishing fetal stem cells from second trimester miscarriages. The second trimester fetal tissue has many advantages. The fetal tissue, especially the hematopoietic tissue, has the optimal characteristics for long term engraftment and regenerative

properties. This is related to a very high number of transplantable primitive cells with high clonogenic and proliferative properties. Furthermore, in contrast to other sources of stem cells described in the prior art U. Boyse et al; U. Broxmeyer et al; U. Kaufman et al; U. Thomson , the fetal cells in the present invention have very low immunogenicity because of the age of the specimen weeks , where the immune system is still poorly developed. The therapeutic mechanism of the fetal stems of the invention, though not fully understood, may be related to cellular reconstitution, replacement of detected cells or enhancement of their function by release of endogenous trophic factors. Tissue derived from second trimester lost pregnancies is completely free from the moral and bioethical problems associated with other fetal or embryonic sources of stem cells because it is considered cadaveric tissue and is fully acceptable for use in therapy. In particular, the present invention relates to the therapeutic use of fetal or neonatal stem cells for hematopoietic or immune reconstitution. Hematopoietic reconstitution with the cells of the invention can be valuable in the treatment or prevention of various diseases and disorders such as anemias, malignancies, autoimmune disorders, and other immune dysfunctions and deficiencies. In another embodiment, fetal hematopoietic stem and progenitor cells and stromal mesencymal cells derived from second trimester weeks gestation miscarriages which contain a heterologous gene sequence can be used for hematopoietic reconstitution in gene therapy. In a preferred embodiment of the invention, fetal bone marrow cells derived from second trimester weeks gestation miscarriages that have been cryopreserved and thawed can be used for cellular reconstitution. In a preferred embodiment of the invention, fetal bone marrow cells derived from second trimester weeks gestation miscarriages can be used for reconstitution directly after testing for compatibility and quality assurance. The invention also relates to methods of collection and cryopreservation of the neonatal and fetal stem and progenitor cells of the invention 4. In a preferred embodiment of the invention, the fetal stem cells derived from second trimester miscarriages can be used in hematopoietic reconstitution. In such an embodiment, the invention provides substantial advantages over the present use of adult bone marrow for hematopoietic reconstitution. Present use of adult bone marrow transplantation is severely restricted by the fact that there is virtually never a perfectly matched genetically identical donor, except in cases where an identical twin is available or where bone marrow cells of, for example, a cancer patient in remission are stored in the viable frozen state in the hope that they will be free of malignant cells and healthy enough to be returned to the patient for treatment of any future relapse. Except in such rare cases, the inevitable genetic mismatch that results can entail the serious and sometimes lethal complications of host versus graft or graft versus host disease. In order to avoid host rejection of the foreign bone marrow cells host versus graft reaction , the patient must be immunologically incapacitated. Such immune incapacitation is itself a cause of serious complications. Furthermore, when and if the donated bone marrow cells become established, they can attack the patient graft versus host disease , who is recognized as foreign. Even with closely matched family donors, these complications of partial mismatching are the cause of substantial mortality and morbidity directly due to bone marrow transplantation from a genetically different individual. In an embodiment of the invention directed to the use of fetal stem and progenitor cells derived from second trimester miscarriages for hematopoietic reconstitution, there are several main reasons for preferring the use of such fetal cells to conventional bone marrow transplantation. First, no donor is required because the cells can be obtained from fetal tissue derived from second trimester miscarriages that would otherwise be discarded. Second, the complications arising in conventional bone marrow transplantation from the need for pretransplantation drug-induced or irradiation-induced immune incapacitation and from acute and chronic graft-versus-host disease are all eliminated because, in this embodiment, fetal stem cells have low immunogeneicity. For these reasons, present restrictions on the use of bone marrow transplantation arising from difficulties in finding even approximately matched donors, and from disease and mortality due to unavoidable genetic incompatibility, do not apply to reconstitution with fetal cells derived from second trimester miscarriages. Furthermore, the prospects of success in bone marrow transplantation decline with age; although it is not clear whether the age of donor or patient is more important, it is proper to infer that younger fetal cells are preferable for hematopoietic

reconstitution. Also, as an example of novel medical applications which may be feasible with fetal cells but not with conventional bone marrow transplantation, restoration with self cells taken at birth can be valuable in the treatment of disorders such as declining immune responsiveness and autoimmunity which occur in increasing frequency with age. Many of the relative disadvantages discussed supra of the use of adult bone marrow cells for hematopoietic reconstitution, also apply to the use of adult peripheral blood for such reconstitution, and thus, the use of neonatal cells for hematopoietic reconstitution according to the present invention provides distinct advantages over the employment of adult bone marrow and peripheral blood stem cells. However, there are possible detrimental effects, known or unknown, of prior chemotherapy or irradiation, on the stem and progenitor cell populations found in these patients. There are additional reasons for preferring the use of fetal cells derived from second trimester miscarriages for hematopoietic reconstitution as provided by the present invention. Fetal tissue from that source is the preferred source of cells for hematopoietic reconstitution, since it is free from viral and microbial agents, known or unknown, latent or otherwise, that may be encountered in later life, other than those transmitted from the mother or during labor and delivery. In addition, in view of the extent to which the hematopoietic stem cell may possibly share with other cells the limitation in total number of cell divisions that it may undergo before senescence, it is proper to assume that the neonatal hematopoietic stem cell has a self-renewal and reconstituting capacity that is at least as great, and perhaps greater, than that of hematopoietic stem cells obtained at any later time in life. The method of the invention may be divided into the following stages solely for the purpose of description: Since fetal hematopoietic cells derived from second trimester miscarriages are envisioned for use in the present invention, descriptions and embodiments of the invention herein described for fetal cells derived from second trimester miscarriages. The Properties of Fetal Tissue Fetal tissues have unique biological and therapeutic properties, which are almost ideal for transplantation, for successful stem cell engraftments, as well as for reconstitution of generally defective cells. Human hematopoietic stem cells are pluriponent with a high capacity to differentiate into the complete repertoire of erythroid, myeloid and lymphoid cell lines.

3 ISOLATION OF STEM AND PRECURSOR CELLS FROM FETAL TISSUE pdf

6: The Human Embryonic Stem Cell and the Human Embryonic | www.enganchecubano.com

Fetal stem cells can be isolated from fetal blood and bone marrow as well as from other fetal tissues, including liver and kidney. Fetal blood is a rich source of haemopoietic stem cells (HSC), which proliferate more rapidly than those in cord blood or adult bone marrow.

Divide the suspension equally into 2 x 50 ml Falcon tubes. Place the fetal liver in a petri dish filled with 20 ml DMEM. Cut liver into small pieces using cell scrapers at room temperature. Mix the suspension well and divide the volume evenly into the prepared Falcon tubes. Add more DMEM to the petri dish to wash down excess tissue and transfer into the same prepared Falcon tube. Grind non-filtered tissue particles with the end of a 10 ml plunger against the mesh. Ensure that there are no remaining clumps. Transfer filtered medium into a fresh 50 ml Falcon tube. Spin at x g for 5 min with a tabletop centrifuge. Add 10 ml of ACK lysing buffer to the pellet and resuspend well. Incubate for 3 min at room temperature. Neutralise the buffer with 10 ml of DMEM. Spin at x g for 5 min at room temperature. CD34 selection Resuspend cell pellet with ml of Robosep buffer depending on the gestation age of the fetal liver and do a cell count using hematocytometer. Mix well and incubate at room temperature for 15 min. Mix well and incubate at room temperature for 10 min. Bring the cell suspension to a total volume of 10 ml with Robosep buffer. Gently resuspend cells before placing the tube without cap into the magnet. Set aside for 5 min. Pick up the EasySep Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labelled cells will remain inside the tube, held by the magnetic field of the EasySep Magnet. Leave the magnet and tube in inverted position for seconds, then return to upright position. Do not shake or blot off any drops that may remain hanging from the mouth of the tube. Remove the tube from the magnet and add 10 ml of Robosep buffer. Gently resuspend the cells and place the tube back into the magnet. Repeat step , and do a total of 4 washes. After the last wash, resuspend cells and combine cells from different tubes with 5 ml of Robosep buffer. Perform a cell count using hematocytometer. Spin cells down at x g for 5 min. Cryopreservation Prepare freezing medium 1. Resuspend cells with freezing medium and aliquot 1. Place cryovials in Mr Frosty and leave it overnight. Transfer cryovials to liquid nitrogen the next day. Incubate at room temperature for 10 min. Dilute the blood sample with equal volume 1: Centrifuge at 1, x g for 30 seconds at RT to allow the Ficoll-Plaque Plus get into the bottom part of the filter in Leucosep tube. Turn Off The Centrifuge Brake!!! And centrifuge at 1, x g for 20 min at RT. Remove the plasma layer and collect the buffy coats layer into a new 50 ml falcon tube. Turn On The Centrifuge Brake!!! And centrifuge for 15 min at x g. Discard the supernatant and resuspend the cell pellet with 10 ml of ACK lysis buffer. Incubate at room temperature for 5 min for RBC to lyse. Centrifuge for 15 min at x g. Resuspend in 1 ml of Robosep buffer and perform cell counting using hematocytometer. CD34 selection and cryopreservation Repeat step 18 to 33 in Part one.

3 ISOLATION OF STEM AND PRECURSOR CELLS FROM FETAL TISSUE pdf

7: Stem cell - Wikipedia

entiation, maturation, senescence, and death.3,4 The discov-ery of resident cells with stem cell characteristics in adult tissues suggests that the heart demonstrates some regenera-tive potential.

Accordingly, also provided in the present invention are methods for monitoring survival, proliferation, differentiation and migration of human cells following transplantation of hNEPs into animal models. For example, survival of hNEPs transplanted into the adult rat brain and their identification can be performed via species-specific antibodies such as antibodies specific for human specific NCAM, GFAP, human nuclear antigen or human mitochondria. Human-specific markers, as described in Table 3, are then utilized to locate and identify transplanted hNEPs. The MAB , a marker specific for human nuclei hNuc , and hMito, a marker for human mitochondria, can be used to identify transplanted hNEPs along the injection tract and in adjacent host tissue. Several other markers can be used to identify subpopulations of the transplanted hNEPs and to reflect the capacity of these cells to retain a progenitor status, as well as to differentiate into neurons and glia. Human NCAM staining is useful in identifying a subset of those transplanted hNEPs that have differentiated along the neuronal-restricted lineage. Human-specific GFAP antibody can be used to identify human astrocytes in situ. In addition, distinctive perikaryal staining can be used to identify cell bodies and process labeling can be used to identify long processes with multiple varicosities. Further provided in this invention are methods for using hNEP cells isolated in accordance with the method of the present invention for transplantation into the central nervous system of animals, including, but not limited to, experimental animal models as well as humans. Until now, human to animal model transplantation studies have been hampered by the limited availability of cross-reactive reagents. However, the ability of isolate hNEPs via the method of the present invention has enabled identification of several markers specific to human cells including antibodies to human nuclei, human mitochondria, human-specific NCAM and human-specific GFAP. Using both lineage-specific and human-specific markers, transplanted hNEPs isolated in accordance with the method of the present invention were demonstrated to survive transplantation in the intact adult rat brain and to have the capacity to generate neurons, astrocytes and a limited number of oligodendrocytes. In the case of hNEP-derived astrocytes, differentiation appeared to be regionally specific. The continued presence of undifferentiated transplanted human hNEPs, identified by the lack of more mature lineage-restricted markers, suggests that early precursors are still present at 28 days following transplantation and are associated with proliferative areas of the intact adult rat brain. In all, undifferentiated and more differentiated hNEP cell derivatives appeared to integrate in a non-disruptive manner into many types of adult brain tissue. However, no evidence of uncontrolled proliferation including masses, heterotopias or tumors were observed. In fact, even the initial injection tract contained only a small number of cells one month after transplantation indicating that cells either migrated away or responded to endogenous signals. Cells preferentially followed three paths when transplanted into the SVZa: Little migration into the hippocampus, cerebellum or cortex was seen. Transplants into the striatum showed much more limited migration, which was primarily astrocytic and involved migration through the parenchyma. Overall these results indicate that hNEPs and their derivatives follow endogenous cues or permissive paths. The ability to travel down the RMS suggests that hNEPs or their derivatives can follow non-radial glial pathways of migration. In addition, mature neurons were observed after only a month following transplantation. These cells clearly matured during this time period, extended processes and appeared to integrate, indicating that transplanting cells into immunosuppressed intact rat brain may be a useful model for studying the developmental biology of human precursor cells, as well as for evaluation of the many potential therapeutic uses of these cells. Finally, while differentiation of hNEPS into oligodendrocytes was minimal, oligodendrocytes were observed along the injection tract, presumably because of local damage caused by the injection. Thus, it is believed that hNEPs will like readily myelinate in an appropriate model and have begun experiments to test this possibility. All of the transplantation studies were

performed with freshly panned and sorted hNEPs. Other animals grafted with unlabeled cells were examined at 28 days using human specific markers. Transplanted BrdU-positive cells were observed in all animals at 2 and 7 days post-transplantation in all graft sites and in the respective injection tracts. Transplanted human NEP cells differentiated into neurons and glia following transplantation into intact adult SVZa and striatum. Grafted cells were examined with both cell type and human specific markers following transplantation Table 3. Soon after grafting 2 days , large numbers of BrdU-labeled cells were observed at both sites. A small number of BrdU-labeled cells stained for GFAP, however, the majority of cells did not react with any of the markers listed in Table 3 indicating that many of the grafted cells remained undifferentiated 48 hours following implantation. The hGFAP-labeled processes appeared much shorter and thicker than any of the endogenous populations of astrocytes as determined by a comparative analysis of the staining pattern obtained with non-species specific polyclonal antisera to GFAP. These results indicate that 48 hours after transplantation, the majority of cells remained undifferentiated, while a small portion had differentiated into astrocytes, but none into mature neurons or oligodendrocytes. At later time points 7 and 28 days , larger numbers of grafted cells stained for neuron-specific and astrocyte-specific markers, while only a small subset of cells labeled for oligodendrocyte markers. These cells possessed unipolar, bipolar and multi-polar processes that in some cases extended hundreds of microns into the cortex, along white matter tracts and into the RMS. Human specific GFAP-positive cells were observed at both graft sites, throughout the ipsilateral corpus callosum extending to the occipital cortex and ipsilateral striatum. At least three distinct phenotypes were apparent, which appeared to be regionally specific. HGFAP-labeled cells in the striatum had a stellate morphology similar to those of the endogenous population, many of which appeared to contact blood vessels. HGFAP-positive cells located along the length of the callosum had long processes similar to the endogenous population of white matter astrocytes, while hGFAP labeled cells located at the wall of the lateral ventricle had processes which extended toward the ependyma in a manner similar to the endogenous population, but with processes that were much thicker. While a significant subset of cells labeled for neuron and astrocyte markers, only a few cells expressed markers consistent with an oligodendrocyte phenotype. Differentiation into oligodendrocytes is believed to be induced by damage from the penetrating stab wound through the white matter. Not all of the transplanted cells labeled with lineage-restricted markers. At 2 and 7 days, significant numbers of BrdU-labeled cells, which double labeled with antisera against human mitochondrial antigens hMito , did not double label with other markers listed in Table 3. Thus, many cells remained undifferentiated. These cells were present at all grafts sites, injection tracts, as well as in the ipsilateral subependymal zone, corpus callosum, and in the ipsilateral RMS. Similarly, at 28 days, a significant number of hNuc-labeled cells did not co-label with other cell type specific markers. However, at day 28, larger numbers of these cells were observed along the wall of the ipsilateral lateral ventricle, in the ipsilateral RMS, and in the corpus callosum extending, in some cases, toward the occipital cortex. The presence of hNEP cells in the subependymal zone of the ipsilateral lateral ventricle and in the RMS at 28 days indicates that a subset of the grafted cells had integrated and continued to proliferate in a manner similar to the endogenous population of precursor cells. To assess whether hNEP cells continued to proliferate following transplantation, two separate experiments were performed. Several pieces of evidence indicate that transplanted HNEP cells continue to proliferate following transplantation into intact adult SVZa and striatum. At both graft sites, the number of BrdU-labeled cells decreased with time from transplantation, while the number and distribution of cells labeled with human-specific antisera increased. In the latter case many hNuc-positive cells remained undifferentiated as determined by a lack of staining with cell type specific or lineage-restricted markers, thus indicating that early precursors continued to be generated over time. At 28 days, BrdU-positive cells were identified near the injection site and along the injection tracts in areas where no BrdU-labeled cells were observed in non-transplanted control animals indicating that cell proliferation continued at the graft site. Lastly, a large number of hNuc-labeled cells that did not co-label with more mature lineage-restricted markers were observed in the subventricular zone suggesting that these cells had integrated and continued to divide in an effort to

function as those previously identified endogenous neural stem cells of the SVZa Doetsch et al. Migration of hNEP cells following transplantation in the intact adult rat brain was region-specific. The distribution of BrdU-labeled cells, as well as those identified with human-specific antisera, indicated that grafted human NEP cells migrated extensively along the ipsilateral rostral-caudal axis. In addition, the distribution of cells over time was graft-site dependent. In SVZa grafted animals at 2 days, intensely BrdU-labeled cells were restricted to the anterior subventricular zone below the corpus callosum within the injection tract, the corpus callosum, and extended medially to surround the rostral and lateral aspect of the lateral ventricle. The nuclei of cells in the corpus callosum were elliptical in shape and oriented along the white matter. These cells displayed an ovoid-shaped nucleus and were aligned along the rostral-caudal axis of the RMS, suggesting rostral migration with features consistent with the behavior of endogenous cells. At 28 days, large numbers of hNEPs labeled with antisera against BrdU, hNuc and hMito were found throughout the subventricular zone and all levels of the RMS extending into the ipsilateral olfactory bulb. In addition, transplanted cells identified with hGFAP immunoreactivity were dispersed along the rostral-caudal axis of the corpus callosum, indicating that cells also had migrated medially and laterally along the white matter, in some cases extending caudally to the occipital cortex. HGFAP-positive cells in white matter exhibited extensive processes similar to endogenous white matter astrocytes. Cells grafted into the striatum displayed a different pattern of migration. Additionally, unlike those cells transplanted in the SVZa, the nuclei of these cells did not exhibit an elliptical morphology. By 28 days, however, transplanted hNEPs were found distributed throughout the ipsilateral striatum, along the more caudal aspects of the corpus callosum, the caudal subependymal zone of the lateral ventricle and posteriorly toward the occipital cortex. The transplanted cells appeared to be migrating in a radial pattern about the site of implantation. The majority of cells were hGFAP positive. In addition, hNEPs could also be found migrating posteriorly along the border of the fimbria and the caudal striatum near the wall of the lateral ventricle. These cells were present in a chain-like fashion with elliptical nuclei oriented in the rostral-caudal axis. Human mitochondrial staining further revealed that these cells had mitochondria-rich processes aligned along the plane of the ventral hippocampal commissure, indicating that these transplanted hNEPs were migrating to the hippocampus. Thus, as demonstrated herein, the hNEPs isolated in accordance with the method of the present invention are useful in developing nonhuman animal models for the study of transplantation of these human cells into the central nervous system. For purposes of the present invention, by nonhuman animal it is meant any animal with a central nervous system comprising a brain and spinal cord. In a preferred embodiment, the animal model comprises a rodent, such as a rat or mouse, a lagomorph, a canine or a primate. However, as will be understood by those of skill in the art upon reading this disclosure, other animals identified as useful in the study of central nervous system cell transplantations can also be used. In the animal models of the present invention, hNEPs isolated from human fetal tissue in accordance with the method described herein are transplanted into either the brain or spinal cord of the animal. The transplanted hNEPS can then be monitored for survival, proliferation, differentiation, and migration via the methods and markers described herein. Expansion and purification of a human-derived multipotent precursor derived from a commercially available source of fetal tissue will also facilitate the development of cell-based therapies for the restoration of CNS tissue function in humans. As demonstrated herein, hNEP cells isolated via the method of the present invention survive, proliferate, migrate and differentiate in vivo in an animal model for transplantation. Based upon these animal experiments, it is expected that the cells will function similarly when transplanted into the central nervous system of humans. Accordingly, the hNEP cells isolated in accordance of the method of the present invention are expected to useful in conditions wherein replacement of neural cells is needed. Several clinical trials with other neural cell types are currently being conducted for these diseases. Similar protocols and procedures used in these clinical trials with other neural cells can be adapted routinely by those of skill in the art for use with the hNEPs of the present invention. The following nonlimiting examples are provided to further illustrate the present invention. These wells were subsequently processed for immunocytochemistry to assess the starting population of Clonetics cells. Unattached cells typically formed

floating spheres. After 24 hours in culture, spheres were removed, gently triturated and re-combined with the attached cells. NEP media was exchanged every other day. Plates were washed twice with DPBS prior to plating neural progenitor cells. After a 30 minute exposure period, unbound cells eNCAM cells were removed and plated onto a dish coated with antibodies to NG2 for 30 minutes. NG2 panning dishes were made by coating dishes with an NG2 antibody 1: Cells were exposed to antibodies to A2B5 1: Antibodies to O4 were used to identify oligodendrocytes in culture. Fixed cultures were stained with antibodies to GFAP to identify mature astrocytes. Example 4 Immunocytochemistry Cultures were stained using antibodies against A2B5 1: Following fixation, cultures were treated with 0. Following 3 washes with PBS, cultures were incubated in the appropriate secondaries 1:

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Nios study material in hindi medium Mel Bochners Via Tasso Project The caring parent Frogs, fleas, and painted cubes Design and Development of Fuzzy-Logic Controllers Deuteronomy and the central sanctuary Microwave cooking recipes in tamil Critical fragments. Why degree is preferable to dichotomy Fodors The Old West Fighting the Greater Jihad Bibliographies (p. [217]-235) The third skill : emotional awareness Avis budget group job application Crime and its consequences High energy nuclear reactions Eight more Canadian poets Medicaid waivers : license to shape the future of fiscal federalism Carol S. Weissert and William G. Weis Travel Narratives from the Age of Discovery Islam (Simple Guide) How to Prepare for the AP Chemistry Antinutrients and phytochemicals in food Act III : Success. Diary of a teenage health freak Management practices and cost management problems in Japanese-affiliated companies in the United Kingdom Great american short stories by wallace mary stegner Computer Aided Engineering Design NFPA electrical inspection manual with checklists Why should I believe the Bible? Experimental study of the influence of environment on animals. Overrunning Germany Strategy and human resource development Tolerance stack up analysis by james d meadows Quantitative Analysis for Management (9th Edition) How to write knockout proposals National reform movements and gifted middle school students Primitivism in modern art Summer at The Cedars Joel H. Johnsons Mormon sawmill Executive development and organizational learning for global business