Cultivation of Human Stem Cells in the Laboratory

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Introduction

One of the science fiction stories for scientists to work on would be treat a cell culture with growth factors and produce any tissue in the body. Today the raw material for such human tissue engineering in the form of universal cell-lines called "Stem Cells* is growing in the laboratory[1].

What are these embryonic stem cells? They are pluripotent cells that can be propagated stably in the undifferentiated state in vitro1 and retain the ability to differentiate into cell types found in an embryonic and adult mice in Vivo[2,3]. They were first isolated from cell masses of mouse embryoblasts in the early 1980's, but have also been derived from other mammals including primates[4].These cells derived from embryonic stem cells have been successfully transplanted into fetal and adult mice where they have demonstrated morphologic and functional integration[4].

Now scientists have achieved a major breakthrough in efforts to culture human stem cells harvested from human embryos[5,6]. The current excitement was touched when Biologist James Thomson and his team at the university of Wisconsin reported that these cells can not only differentiate into all types of tissue of the human body butt under carefully controlled conditions, be maintained continuously as undifferentiated cells in laboratory cultures[7-9]. This new discovery will open the way to study various problem in early development of human embryos[10]. It can be used to grow replacement tissue like dopaminergic neurons for patients with Parkinson's disease, heart muscle cells to repair damaged hearts, or insulin producing cells for diabetic patients[6,11]. These developments however would require the researches to identify the signals that tell a stein cell to become one tissue or other [6,12,14] James Thomson who began working with embryos from rhesus monkeys 5 years ago, stimulated cells from days old human embryos[15,16] called blastocysts to grow on a Layer of mouse feeder cell in a lab dish[7]. (Many researchers reached up to this). But Thomson went further and coaxed these stein cells to grow in five immortal cell lines[5]. Immortality was depicted by the presence of telomerase which repairs frayed chromosome ends[12,13]. A thousand miles away in Baltimore, Gearhart of John Hopkin's University was isolating similar cells by culturing fragments of human fetal ovaries and testes[17,18], Related studics were being carried out in California at Geron Corporation in Mento Park and in Roger A Pcderson Research Laboratory at the University of California at San Francisco[16]. Pera at Monash University in Clayton, Australia together with scientists at the Hadassa Medical Center in Jerusalem and the National University of Singapore achieved extensive serial cultivation of cells from human blastocysts[16]. Smith and his team at Edinburgh in Scotland has been trying to develop a human stem cell line too but have nothing to announce yet[16].

Directing Development

Thomson[6] also demonstrated that after undifferentiated proliferation in vitro for 4-5 months these cells still maintained the development potential to form.trophoblast and derivatives of all 3 embryonic germ layers including gut epithelium (endodenu) cartilage and bone, smooth muscle and striated muscle (mesoderm). Similar results were obtained when these cells were transplanted under the skin of an immunocompromised mice producing teratomas. Histology revealed derivation from all the three embryonic germ layers (ectoderm, mesodenn and endodenu). How is it possible to induce embryonic stem cells to mature into desired tissue? The answer to this question will be the focus of future scientists[16]. Bain and Golileib mid their associates at the Washington University School of Medicine

have shown that treating mouse embryonic stem cells with vit A retinoic acid can stimulate them to produce nerve cells. Firpo and her co-workers in Gordon Kellers Laboratory at the National Jewish Medical and Research Center in Denver had comparable results in deriving blood cells[19]. They discovered certain growth factors which stimulated cells derived from embryonic stem cells to produce complete range of cells found in blood[20]. Field and his associate at Indiana University School of Medicine have been able to yield cardiomyocytes of greater than 99% purity[16], To achieve this goal, they first introduced an antibiotic resistance gene into mouse embryonic stem cells. The gene had been engineered to express itself only in cardiomyocytes[21], After allowing the cells to differentiate and exposing them to enough antibiotics to kill cells that lacked the resistance gene they were able to recover essentially pure cardiomyocytes[22]. Researchers have also identified a growth factor with an EGF Jomain Criptol (Cr-I) in cardiac differentiation[23]. During embryonic development Cr-I is expressed in the mouse blastocyst primitive streak and later is restricted to developing heart[23]. Likewise Deacon of Haward Medical School and his co-workers have transplanted ES cells into a particular region in the brains of adult mice[16,23]. The cells assumed the typical shape of neurones. Some of these cells produced an enzyme that is needed to make the neumtransmitter dopamine arid occurs in large quantity in dopamine secreting neurons[16,23]. Whether such cells look normal and also function normally has not been assessed[16]. But the results from the mouse studies suggest that as researchers gain experience with human ES cells it will become possible to stimulate them to produce at least blood cells, heart muscle cells and neurons[16]. Other medically valuable types might be achievable such as pancreatic cells for treatment of diabetes[24], skin fibroblasts for treatment of bums or wounds[16]. Recently scientists have identified only a handful of the biochemical signals that dictate the differentiation of embryonic stem cells and progenitor cells into specialized cell types[25,26].

Legal Questions Raised

The full potential of recent discoveries on embryonic stem cell will be realized only if society deems this research worthy of support[27,29]. Many people feel that human embryos growing in the laboratory dishes even at the earliest stages of development wan-ant special moral consideration because they can grow into human beings if returned to uterus for gestation. (NIH tried to ban the stem cell research according to public law 1 05-78 section 513(a) USA). But Director Harold Vennus of NIH sought legal counsel and NIH finally approved that embryonic stem cells will be exempted from the law as they cannot grow into embryos. Researchers remove the outer layer of cells in the originating blastoc st. These are essential for the development of placenta which normally nourishes the product of conceptiont[16]. By taking away inner cell mass layer it is not possible for the rest of the cells to develop in a uterus. Thus embryonic stern cells do provide a source of medically useful differentiating tissue that lack the awesome potential of an intact embryo[16]. These cells also have a darker side. The jumble of cell types they form when injected into mature mice constitute teratoma[6]. Researchers will have to be sure before using these cells therapeutically that they all have differentiated enough to be incapable of spreading in approximately or forming unwanted tissue. All the differentiated cells would probably be useful as isolated cells or as suspension. For would be tissue engineers learning how to direct plueripotent stem cells towards building entire organs will be hugely difficult. But some researchers are working on these problems also[6,30].

Benefits and Uses

Human embryonic stem cells should offer insight into developmental events that cannot be studied directly in the intact embryo but have important consequences in clinical areas like birth defects, infertility and pregnancy loss. Another benefit would be that researchers might be avoided another potential of human ES cells is in the pharmaceutical industry. The availability to normal human cells of virtually any type can be used for drug research and development. Currently cells lines used for

screening are derived from animals or tumor cells[16].

Questions to he Answered

Many questions related to the possible therapeutic use of human embryonic stem cells have riot been assessed in mouse ES cells. Using knowledge of our understanding of the molecular pathways and the molecules that mark specific of differentiation cell types the following question can be answered.

1. Can human ES Cells be forced to differentiate along desired pathway?
2. Can we make all ES Cells in a culture simultaneously develop along that pathway?
3. What exactly are the intermediary cell types?
4. What markers and which methods to be used to set out the desired pathway?

As research on such cells could provide insight into fundamental questions that have puzzled embryologist for decades. Understanding these processes in our own species will ultimately provide us with greatest benefits and deepest satisfaction.

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