

Biomedical Pathways

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Michigan Research Today: Stem Cells

Stem cells represent both the brightest hope for some patients and the ultimate challenge for scientists. The potential of these cells to replace damaged tissues and organs holds great promise for patients with debilitating illnesses such as Parkinson's, diabetes, and spinal cord injuries. Investigators at U-M are studying the basic biology and therapeutic potential of stem cells.

The University of Michigan Human Embryonic Stem Cell Center was launched in 2002 with funds from the Endowment for the Basic Sciences and is affiliated with the Center for Organogenesis. In 2003 the University of Michigan Medical School was designated one of three National Institute of Health "Exploratory Centers for Human Embryonic Stem Cell Research" with a \$2.3 million grant from the National Institute of General Medical Sciences (and with another year of funding awarded this year). The Center supplies U-M investigators with a local source of approved human embryonic stem (hES) cell lines and the equipment and training needed to use them in research.

A challenging aspect of stem cell biology is understanding how embryonic stem (ES) cells differentiate into specialized cells and how differentiation can be directed so desired cells and tissues can be produced. Although ES cells in culture differentiate spontaneously, introducing various biological agents into stem cell cultures can provide an opportunity to halt differentiation, redirect the process, and then resume an alternate differentiation pathway. Exposure to a particular growth factor(s) encourages differentiation into one or two specific cell types associated with that growth factor. Another way to manipulate differentiation is to overexpress a gene that contributes significantly to the development of a specific cell type. Ultimately, scientist may be able to coax ES cells to even produce desired organs and tissues. The U-M Human Embryonic

Stem Cell Center provides funding for pilot projects, many of which focus on how ES cells differentiate into a variety of cell types including, bone, neurons, and blood cells.

At the opposite end of the spectrum, the unregulated reproduction of stem cells may be involved in cancerous tumor growth. Tumors are composed of a diverse population of cells; however only a small minority of cells is able to grow new tumors and give rise to all of the different kinds of cells found in a tumor. A small population of cells expressing surface protein markers common to stem cells and exhibiting many of the properties of stem cells have been found in breast cancer tumors by University of Michigan researchers Michael Clarke, MD, Max Wicha, MD, Muhammad Al-Hajj, PhD, and Sean J. Morrison, PhD. This finding has led other researchers to investigate whether cancer stem cells might be present in all cancerous tumors. Investigators have found stem cells in brain cancer and multiple myeloma, and will continue looking for stem cells in other types of cancer, such as prostate, head and neck, skin, lung, and pancreatic cancers, as part of a major research initiative in the Cancer Center over the next five years. U-M researchers working with stem cells have formed a seminar series to collaborate and share information about the role of these cells in different types of cancer.

In our inaugural issue of Biomedical Pathways we feature two U-M investigators, Sue O'Shea, PhD, Director of the Exploratory Center for Human Embryonic Stem Cells and Max Wicha, MD, Director, Comprehensive Cancer Center. Their research endeavors have opened up new pathways in our understanding of the basic biology of cellular differentiation as well as the pathogenesis of cancer.

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Tapping the Potential of Human Embryonic S

Emryonic stem (ES) cell research has tremendous promise for directing undifferentiated cells to form the specialized cells required to replace cells lost following disease, injury, or normal aging. Despite the potential, stem cell research may be one of the most hotly debated topics in the history of science. Stem cells, whether derived from the inner cell mass of the pre-implantation blastocyst from fetal tissue or taken from adult tissues, have caught the imagination of both scientists and the public.

ES cells are derived from the inner cell mass of the blastocyst four to five days after fertilization, and prior to implantation of the embryo in the uterine wall (Figure 1). Because the inner cell mass forms the embryo itself, ES cells are capable of producing differentiated cells of all tissue, blood, muscle, bone, and neural cell types in vivo, from all three early germ layers (endoderm, mesoderm, and ectoderm). ES cells were initially derived from mouse blastocysts in the early 1980s, and were employed extensively to create mutant mice by gene-targeting. There was considerable interest in deriving ES cell lines from other species, and Jamie Thomson at the University of Wisconsin, using techniques developed to derive primate ES cells, first successfully cultured human ES (hES) cells in 1998. A number of other hES cell lines were derived by scientists at Technion University in Israel, at the University of Georgia, UCSF, at Monash University and in Seoul, Korea; these are the cell lines approved for NIH funded research. Since that time, scientists worldwide have developed approximately an additional 100 lines of human embryonic stem cells.

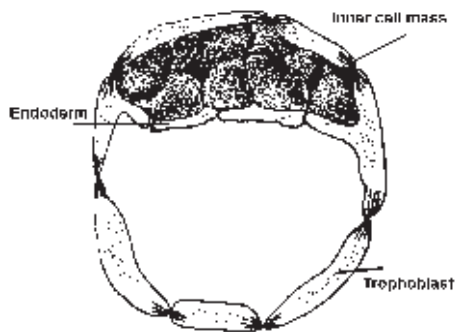


Figure 1

Schematic representation of a day four post-fertilization blastocyst. The outer layer of trophoblast cells will implant into the endometrium of the uterus and form the placenta. The inner cell mass will differentiate into all the tissues of the embryo itself. To derive embryonic stem cells, blastocysts at this are placed into tissue culture, and the trophoblast removed. Cells from the inner cell mass divide rapidly and are sub-cloned to form lines of embryonic stem cells.

In 2001, the Michigan Center for Human Embryonic Stem Cell Research was established here at the University, initially by funds from Dean Lichter's Endowment for the Basic Sciences, followed by funding of an NIH Center Planning Grant in 2002. This was one of three Center awards made by the NIH; the other two grants went to the University of Wisconsin, and to scientists at the Fred Hutchinson Cancer Center in Seattle. The goals of the

centers are to provide training in the culture of these cell lines, and educate scientists and students in their use and characteristics. What is unique about the Michigan Center is that its home is in the Center for Organogenesis, where developmental biologists and adult stem cell biologists can find support to translate their research to using the hES cells. To date, the hES Center has funded nine pilot projects (\$80K each), and will fund an additional 3-5 projects in the next year.

Stem cells are unique in that they have the ability to self-renew as well as to differentiate into multiple types of cells. ES cells have a unique cell cycle characteristics (attenuated G1), and high levels of telomerase activity, which may explain their ability to grow indefinitely in culture. Importantly, ES cells can be successfully grown from a single cell, and resulting cell lines maintain the characteristics of the parent cell. In culture, they must be carefully maintained to inhibit premature differentiation. For mouse ES cells this is done by simply adding leukemia inhibitory factor (LIF) to the culture medium, but human ES cells are kept from differentiating by growing them on primitive fibroblast cells, derived initially from mouse embryos. As scientists learn more about the biology of the hES cells, it should be possible to identify the critical factors produced by the fibroblasts that inhibit hES cell differentiation. Dr. Gary Smith's laboratory is using bioartificial substrates and microfluidics to study the growth factor requirements of the hES cells, and a group led by Dr. Gil Omenn, Dr. Phil Andrews, and Dr. John Strahler is using proteomics to better understand the basic biology of the hES cells.

In addition to providing cells for tissue engineering approaches, the human ES cell lines may provide alternatives to animal testing for toxicological and pharmaceutical development, provide a way to study disease progression, and offer developmental biologists the opportunity to study the very earliest stages of development of the human embryo. Despite the interest in understanding how various cell types form, it is quite difficult to control the formation of a desired cell type, and considerable research here and elsewhere is directed at understanding how to form, for example, a beta cell that would produce insulin in response to glucose signaling for the treatment of diabetes.

There are several ways in which ES cell differentiation can be influenced: one is by exposing the cells to growth factors that promote the formation of the desired cell type. Research being done in Dr. Mark Russell's laboratory uses this approach to develop cardiac myocytes from human ES cells, with the hope that they will eventually be used to replace damaged heart tissue. This approach can be particularly powerful when the growth factors that are present in the developing tissue have been identified. When those growth factors are not yet known, another approach is to grow the ES cells in co-culture with embryonic tissue, so that the unknown growth factors produced by the surrounding tissues can guide differentiation. Two groups in the UM hES Center are taking this approach. Dr. Doug Engel's lab is differentiating erythroid progenitor cells, and Dr. Paul Krebsbach and Dr. Peter Ma's labs are producing bone cells using this approach.

Another way that ES cells can be promoted to differentiate into the desired cell type is by altering the genetic constitution of the ES cell. Thus, by “forcing” cells to express a gene restricted to the desired cell type, and then selecting cells that express the transgene using either flow cytometry or antibiotics, relatively pure types of cells are produced. These uniform populations of cells can be studied in their own right, can be further differentiated using growth factors, or can be implanted into models of disease. Dr. Jack Parent, who hopes to transplant neuronal precursor cells in models of experimental stroke, is taking this approach to differentiate neuronal cells from the hES cells. By transfecting hES cells with a gene restricted to primitive neurons, and then selecting cells that express that gene, they hope to develop relatively pure cultures of neurons for transplantation and eventual tracking in the brain. The O’Shea lab takes this approach both to develop neuronal cells from ES cells, but also because it allows them to study the function of a gene in the differentiation of a totally undetermined cell type—in that way it provides a model to study gene function during an otherwise inaccessible period of development. The ability to rapidly down-regulate genes involved in early development using shRNA technologies has also been employed in hES cells to obtain different cell/tissue types, and to study the role of that particular gene in development.

In addition to funding pilot research projects, the hES Cell Center provides training in hES cell culture, organizes graduate courses in stem cell biology—this year on the mesenchymal stem cell—and funds graduate student travel. The hES center provides RNA and DNA from differentiated cells so that investigators can determine if a gene of interest is expressed during differentiation, provides MEFs, conditioned culture medium and other supplies. In addition, community outreach and education in stem cell biology for the public is an important mission for the center.

Stem cell research has the potential to globally impact human health—spanning the spectrum from birth defects to aging disorders. Stem cell differentiation is one of the hallmarks of human embryonic development, and understanding the mechanisms of normal and aberrant cell division and differentiation can lead to better diagnosis, prevention, and treatment of birth defects that affect millions of children born each year. In addition, when scientists can reliably produce specific healthy cells and tissues, debilitating conditions such as stroke, heart disease, diabetes, osteoporosis may be more effectively treated. ES cells could also deliver replacement genes, growth factors or pharmaceuticals to damaged tissues. With continued effort, resources and progress, the potential applications of this technology are vast.

Sue O’Shea, Ph.D. a professor of cell and developmental biology, is P.I. on the NIH grant and director of the center.



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U-M Scientists Find “Stem Cells” in Human Breast Cancer

In breast cancer, tumors are comprised of malignant cells that all have the ability to generate tumors—or so researchers believed, until recently. At the University of Michigan, investigators Muhammad Al-Hajj, Max S. Wicha, Adalberto Benito-Hernandez, Sean J. Morrison, and Michael F. Clarke have discovered that only a small number of cells (as few as 100 within a tumor mass) are tumorigenic. The remaining cells are non-tumorigenic cells that have little proliferative ability and do not cause metastasis if they migrate from the primary tumor site.

These cells exhibit typical features of stem cells—highly proliferative, self-renewing, long-lived, and able to direct cell differentiation into a variety of cell types (pluripotent). Breast cancer “stem cells” have been characterized as a mammary stem cell that has undergone mutation from genetic instability or damage from environmental insult such as radiation, which can occur as a result of a time-sensitive exposure (during late adolescence, when the mammary gland may have the highest number of stem cells) or over time as a consequence of repeated exposure to toxic events. Mutational alterations in these cells compromise regulatory mechanisms that inhibit unrestrained growth, leading to malignancy. Identified and labeled as CD44⁺CD24^{-/low}Lineage⁻, these cells are largely chemotherapy-resistant and self-protective due in part to higher expression of genes for Bcl-2, BCRP (breast cancer resistance protein) and MDR (multidrug resistance transporter-1, which confers drug resistance) making them difficult to kill with conventional treatment protocols. As few as 100 cells of this type consistently created tumors in NOD/SCID mice, both after initial implantation with tumor stem cells taken from primary or metastatic tumors in breast cancer patients and in subsequent serial passage. Conversely, bombarding the mammary glands of mice with the distinct phenotypes of the tumorigenic cells from the same tumor did not result in new tumors.

Cell surface markers enabled researchers to distinguish between tumorigenic cells and the phenotypically distinct non-proliferative tumor cell that a cancerous stem cell produces. Specimens of tumor cells were collected from nine patients, one from a primary cancer and the remaining from metastatic plural effusions. Flow cytometry was used to segregate cells that were positive or negative for each marker from passage T1 or T2 cells. The cell surface markers CD44 and CD 24 (adhesion molecules), and the breast /ovarian cancer specific marker B38.1 were used to identify which cells types were most likely to generate tumors. After injecting mouse mammary fat pads with tumor cells, the CD44⁺ cells (8/8), the B38.1⁺ (8/8), and the CD24^{-/low} cells (12/12) all generated visible tumors within 12 weeks post-injection. No CD44⁻ (0/8) or B38.1⁻ (0/8) cells generated tumors, although two of 12 mice injected with CD24⁺ cells demonstrated small tumors on necropsy. This was attributed to the 1-3% rate of contamination from CD24^{-/low} cells in the sample. Since CD44 and B38.1 markers were consistently expressed simultaneously from the same cells, subsequent investigations focused exclusively on CD44 and CD24 expression markers.

Lineage markers associated with normal cells types, such as CD2, CD3, CD10, CD16, CD18, CD31, CD64, and CD140b, were not expressed in cells from the tumors samples taken from patients. This meant that Lineage⁺ cells from unpassaged or early passaged tumor cells, and other normal cell types such as human leukocytes, endothelial cells, mesothelial cells, and fibroblasts, could be excluded from subsequent investigation. Lineage⁻ tumor cells consistently appeared neoplastic upon examination, in both tumorigenic and non-tumorigenic cell masses, with large nuclei and prominent nucleoli (Figure a, b). When the injection sites of 20,000 CD44⁺CD24^{-/low}Lineage⁻ tumorigenic cells were compared with 20,000 CD44⁺CD24^{+/low}Lineage⁻ sites six months post-injection, none of the CD44⁺CD24^{+/low}Lineage⁻ contained detectable tumors, but the CD24⁻ sites contained tumors of about 1 cm, with malignant cells confirmed using hematoxylin and eosin-staining.

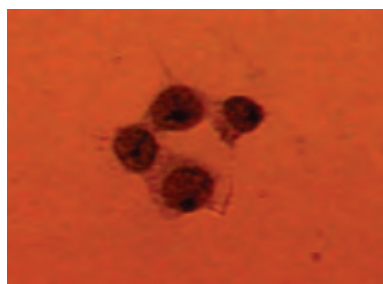


Figure a

This image shows cancer stem cells isolated from human breast cancers by U-M scientists. Photo credit: Courtesy of Proceedings of the National Academy of Sciences.

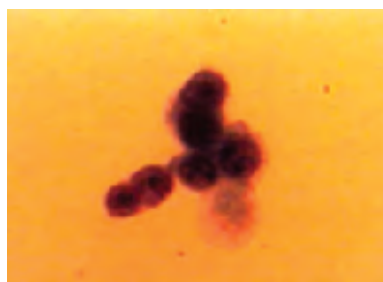


Figure b

This image shows cells from human breast cancers which have lost the ability to spread and form tumors. Photo credit: Muhammad Al-Hajj, University of Michigan. Courtesy of Proceedings of the National Academy of Sciences.

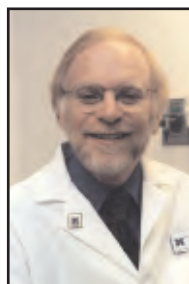
An important characteristic that tumorigenic stem cells share with normal stem cells is their ability to reproduce phenotypically diverse populations of cell types. CD44⁺CD24^{-/low}Lineage⁻ cells always produced heterogeneous cell populations of tumor-producing and non-tumor-producing cells, even when serially passaged, while CD44⁺CD24^{+/low}Lineage⁻ cells never produced cancer stem cells. Additionally, tumorigenic cells undergoing serial passage did not lose their ability to produce cancerous tumors regardless of the number of passages. The stability of the cell aberration that results in malignancy suggests that these cells follow a similar path in self-renewal and differentiation that occurs in normal ES cells.

Identification of these tumor stem cells as the culprits in the formation of aggressive metastatic cancer has broad implications in the way cancer is currently treated by physicians. If only cancer stem cells have the capacity to

repopulate tumor mass after treatment, they would make the ideal and specific targets for the development of anti-tumor drugs. This is a novel concept in rational drug design of anti-cancer therapies.

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Muhammad Al-Hajj, Max S. Wicha, Adalberto Benito-Hernandez, Sean J. Morrison, Michael F. Clarke. Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences, 2003; v. 100:7:3983-3988.



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