Sources of Genetic Instability in Human Embryonic Stem Cells.

Grant Type: SEED Grant

Grant Number: RS1-00428

Human embryonic stem cells (hESCs) originate directly from human embryos, whereas induced pluripotent stem cells (iPSCs) originate from body (somatic) cells that are re-programmed by producing or introducing proteins that control the process making specific RNAs. Together, both these pluripotent cell types are referred to as human pluripotent stem cells (hPSCs). Several reports have observed that in hESCs grown for long times, their genetic material, DNA, is unstable. The stable maintenance of DNA is performed by groups of proteins functioning in different systems globally known as DNA repair pathways. Since the development of aneuploidy is closely linked to cancer and to deficiencies in DNA repair, we have studied the propensity of hPSCs to repair their DNA efficiently by 4 major known DNA repair pathways. In addition, we are also investigating if specific damage to DNA in either hPSCs or somatic cells is processed differently and could lead to deleterious mutations. One major goal of the CIRM SEED grant mission is to bring new researchers into the hPSC field. The results we obtained during the funding period indicate that we have succeeded in that objective, since initially our laboratory had little experience with hESC culture. However, through courses and establishing critical collaborations with other hESC laboratories, we developed expertise in hPSC culture techniques. Most conditions for hPSCs growth require cells (feeder cells) that serve as a matrix and provide some factors needed for the pluripotent cells to divide. In accomplishing this aim, we perfected a method to generate reproducible feeder cells that significantly reduces the time and cost of feeder cell maintenance, and also developed a non-enzymatic and non-mechanical way to expand hPSCs. We now have experience with at least 5 hPSC lines and have methods to introduce foreign DNAs into hESCs and iPSCs to monitor DNA repair in hPSCs. In Aim II of our grant, we used our accumulated knowledge of hPSCs and DNA repair to investigate 4 DNA repair mechanisms in hPSCs and in somatic cells. Depending on the DNA damage, there is often a preferred DNA repair pathway that cells use to alleviate potential harm. We initiated our investigation by treating hPSCs using different DNA damaging agents, including ultraviolet light and gamma radiation. However, we found that hPSCs exposed to these agents rapidly died compared to treatments that allowed somatic cells to continue growing. Therefore, we developed methods to study DNA repair in hPSCs without directly treating the cells with external agents. We treated closed, circular DNA (plasmids) with damaging agents separately, outside the hPSCs and then introduced them into the hPSCs. The plasmid DNA has a sequence that codes for a protein that is produced only when the damage is repaired. The length of time for repair both in hPSCs and in somatic cells was followed by determining the protein production. We have shown superior DNA repair ability and elevated protection against DNA damage in hPSCs compared to somatic cells for ultraviolet light and oxidative damage, two common sources of damage in cells. A major pathway for joining double-strand DNA breaks in mammalian cells, non-homologous end-joining (NHEJ) repair (error prone), is greater in H9 cells than in iPSCs. Another way to repair double-strand DNA breaks that uses similar (i.e., homologous) sequences is lower in iPSCs compared to hESCs and somatic cells. Further study of these repair pathways is warranted, since several methods can be used to form iPSCs. Therefore, the genomic stability for iPSCs could depend on the method used for their generation. DNA repair analysis is critical to understanding how hPSCs protect against damage, but if left unrepaired, cells can turn damage into mutations when the damage is copied by enzymes (DNA polymerases) before repair occurs. Therefore, to monitor the mutations that ultimately lead to cancer or alter hPSC biology, we are using a plasmid that is damaged outside the cells and will make copies in hPSCs and somatic cells. That plasmid is introduced into cells and then the copies are recovered. The number of mutations found in the plasmid DNA indicates the likelihood of observing mutations in hPSCs compared to mutations in somatic cells. Together, these results will yield data on the stability of hPSCs and also a basis to monitor cells for stability which could serve as an indicator of safety for clinical use.
The constant exposure of cells to endogenous and exogenous agents that inflict DNA damage requires active repair processes to eliminate potentially mutagenic events in stem cells leading to cancer. The same agents menace early human embryos with DNA damage that can ultimately lead to mutations, cancer, and birth defects. In vitro, human embryonic stem cells (HESCs) spontaneously undergo events leading to genetic instability and mutations. All these three types of genetic problems can have similar links to malfunctions in DNA repair systems, but little information now exists for HESCs. Therefore, the first step in understanding the causes of HESC genetic instability is to understand which DNA repair systems are defective. We will investigate the basis for this phenomenon in HESCs by evaluating their capacity to either repair DNA or form mutations. First, we will culture two HESC lines and compare HESC repair and mutation formation to that of control cells. We will use a new technique which simplifies the production and use of the feeder cells that support the growth of the HESCs. We will also test the genetic stability of HESCs grown on conventional feeder cells, as well as those grown in feeder free culture. We will use three types of DNA repair assays to monitor the genetic stability of the two HESC lines grown in these different ways. In the first of these assays, DNA molecules with different randomly-induced damage are transferred into HESCs, and DNA repair is followed by the re-establishment of the activity of a reporter protein that is coded for in the damaged DNA. A second assay will introduce specific DNA damage at a unique site in DNA that is transferred to HESCs and repair is determined using a polymerase chain reaction-based technique. Since aneuploidy is also known to be caused by double-strand DNA breaks, we will use two other assays to evaluate capacity of HESCs to repair that type of damage. These experiments will indicate if DNA repair pathways that eliminate DNA damage are dysfunctional and cause genetic instability. The final endpoint for these preliminary experiments is the formation of mutations. To study this, we have modified an assay system so that it will function in normal human cells to monitor mutations which arise spontaneously or those which are induced by various agents. In summary, these investigations will provide the basis for understanding genetic instability in HESCs that can direct cells to tumorigenic outcomes. The employment of HESCs clinically will require such knowledge. Moreover, these results will also yield information on susceptibility to mutations of cells early in development. The practical and basic science aspects of this seed grant proposal should lead to a complete proposal in the near future.
Statement of Benefit to California:

Human embryonic stem cells (HESCs) hold the potential to cure or alleviate many chronic illnesses, including cancer, but an immense gap exists between the achievement of the goals of stem cell based medicine and the current state of the art. Several stages of development including the following are required:

1. Routine, standardized, simple protocols for the indefinite growth of HESC in the normal, undifferentiated state, in completely defined medium.
2. Control over differentiation of the cells in (1) to all adult cell types of interest.
3. Control over the maintenance of the differentiated state of derivatives of (1), in sufficient complexity to recreate normal functional histology.
4. Techniques, therapies, and protocols that allow immune tolerance of regenerated tissue, without rendering the human recipient immunodeficient.

Researchers are still struggling with steps 1 and 2. Although claims of feeder cell and animal product-free, long-term, undifferentiated HESC culture have been made, this is not the current state of the art in laboratories. These claims may be fortuitous or true for only a few HESC lines. The public anticipates a quick success of human stem cell technology and application to human disease, but the promise of stem cell therapy requires basic scientific work that is critical, but may not make headlines. Imprudent claims of miraculous cures could dim public enthusiasm. Few if any data exist regarding DNA repair systems or mutation frequencies of HESCs. We propose to investigate mechanisms underlying genetic instability in cultured HESCs. This instability limits HESC research and therapeutic applications. The data generated by this research according to Proposition 71 will be of lasting value to the People of the State of California for the following reasons:

1. This proposal focuses on a serious, basic difficulty with respect to the growth of undifferentiated HESCs that is a barrier to their human therapeutic use.
2. In the future, if the focus of the stem cell field shifts to the as yet unavailable somatic nuclear transfer (SNT) methods, this proposed research, will provide a basis for the comparison of HESCs and SNT cell lines.
3. All humans begin as embryonic stem cells, therefore data generated by the proposed research will impact maternal health, well baby programs, early childhood development/learning, etc., because mutations are involved in birth defects as well as cancer. Therefore, understanding the causes of mutations in HESCs could assist in avoidance or reduction in birth defects that would aid both the families and the government of California.
4. All of the work described in this proposal will be conducted by individuals in California and most probably will result in the hiring of a graduate of a California institution of higher education, thus reducing unemployment and helping educate a new generation of California researchers in HESC use.