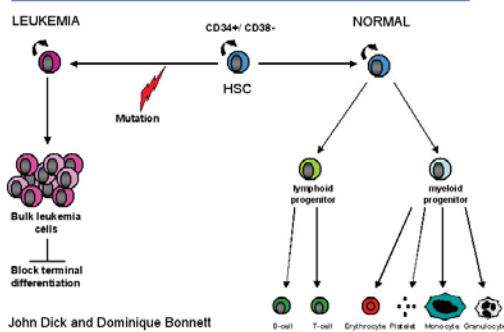


Leukemia is arranged as a hierarchy similar to normal hematopoiesis



John Dick and Dominique Bonnett

Figure 1. Development of Normal and Cancer Hematopoietic Stem Cells.

Human hematopoietic cells are organized in a hierarchy that is sustained by a small population of self-renewing hematopoietic stem cells (HSCs). HSCs give rise to progressively more lineage-restricted, differentiated progenitors with reduced self-renewal capacity, which in turn produce functionally mature blood cells. Disruption of pathways regulating self-renewal and differentiation through the acquisition of transforming mutations generates cancer stem cells capable of sustaining growth of the leukemia/lymphoma clone.

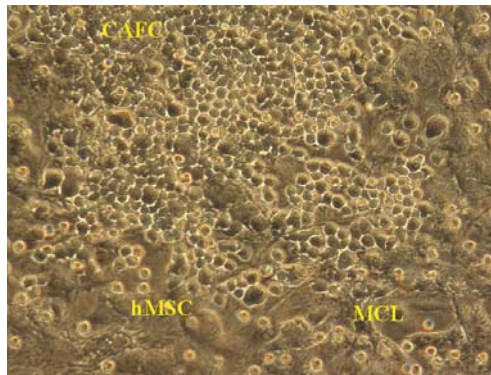


Figure 2. MCL Cells in Long-Term Culture

MCL stem cells (round dark cells) cluster in cobblestone areas (CAFC), and MCL cells (round bright cells) on human stromal cells (flat dark cells) (hMSC)

medicine at RWJMS and director of hematologic malignancies at CINJ; Tulin Budak-Alpdogan, MD, associate professor of medicine at RWJMS and CINJ; Lauri Goodel, MD, RWJMS associate professor of pathology and laboratory medicine; and Hana Aviv, PhD, RWJMS associate professor of pathology and laboratory medicine. 🐶

of developing useful strategies that will document the safety of these cells for therapeutic use.

Humans are large bodied animals with a long life span. We start our lives as a single cell, but when we achieve our adult size and maximal tissue complexity, our bodies are composed of roughly 16 trillion cells that fall into approximately 500 different categories of cell types. This is a remarkable feat by any standard; especially considering that the entire human genome that encodes everything we have been, are, and will be must be completely and accurately duplicated in our cells with each mitotic division. However, the mitotic demand on our cells does not stop when we achieve full size at adulthood. Because the life span of our body is much longer than that of most of its individual constituent cells, an efficient system of cell renewal and replacement has evolved. Examples of this are found on the surfaces of our skin and intestinal lining, where older cells are constantly being shed and replaced by new cells. Individual cells in other organs, such as the kidney, liver and lungs, also eventually wear out and must be continually replaced during normal aging. In addition, we sustain injuries in the form of cuts, abrasions, bruises and broken bones. Here, not only must damaged or dead cells be replaced, but new cells must migrate into the damaged region, repair the tissue architecture and restore function. Alternatively, the damaged tissue can be “patched up” and regions containing dead cells replaced with a cell-derived “bandaid” called scar tissue.

These remarkable feats are accomplished, in large part, through the activity of a relatively small number of widely-scattered, self-renewing cells called stem cells. These cells have the ability to proliferate as needed and give rise to rapidly dividing progenitor cells whose progeny subsequently develop into spe-

cific lineages of differentiated cells.

Embryonic stem cells (ESCs), derived from early human embryos, have an apparently unlimited growth potential and capacity to differentiate into almost any cell type, but their bane is a tendency to unpredictably develop into cancers *in vivo*

— a property that may seriously limit their safe clinical use. By contrast, adult stem cells, such as mesenchymal stem cells (MSCs), avoid ethical concerns, but these cells are more difficult to propagate *in vitro* and show a more restricted differentiation potential than ESCs. MSCs can be isolated from umbilical cord blood and, as described in more detail by my collaborator, Dr. Biaggio Saitta, in this issue, can differentiate into bone, cartilage, muscle and connective tissues.

Ensuring the safety of stem cell therapy

by Robert G. Nagel

Adult stem cells are rare cells scattered throughout our bodies that, when called into action, have the ability to proliferate and give rise to progeny that can differentiate into a number of different cell types. Mesenchymal stem cells (MSCs) are one type of adult stem cell that can be isolated from umbilical cord blood. After their isolation, MSCs must be extensively expanded in cell cultures in order to generate numbers

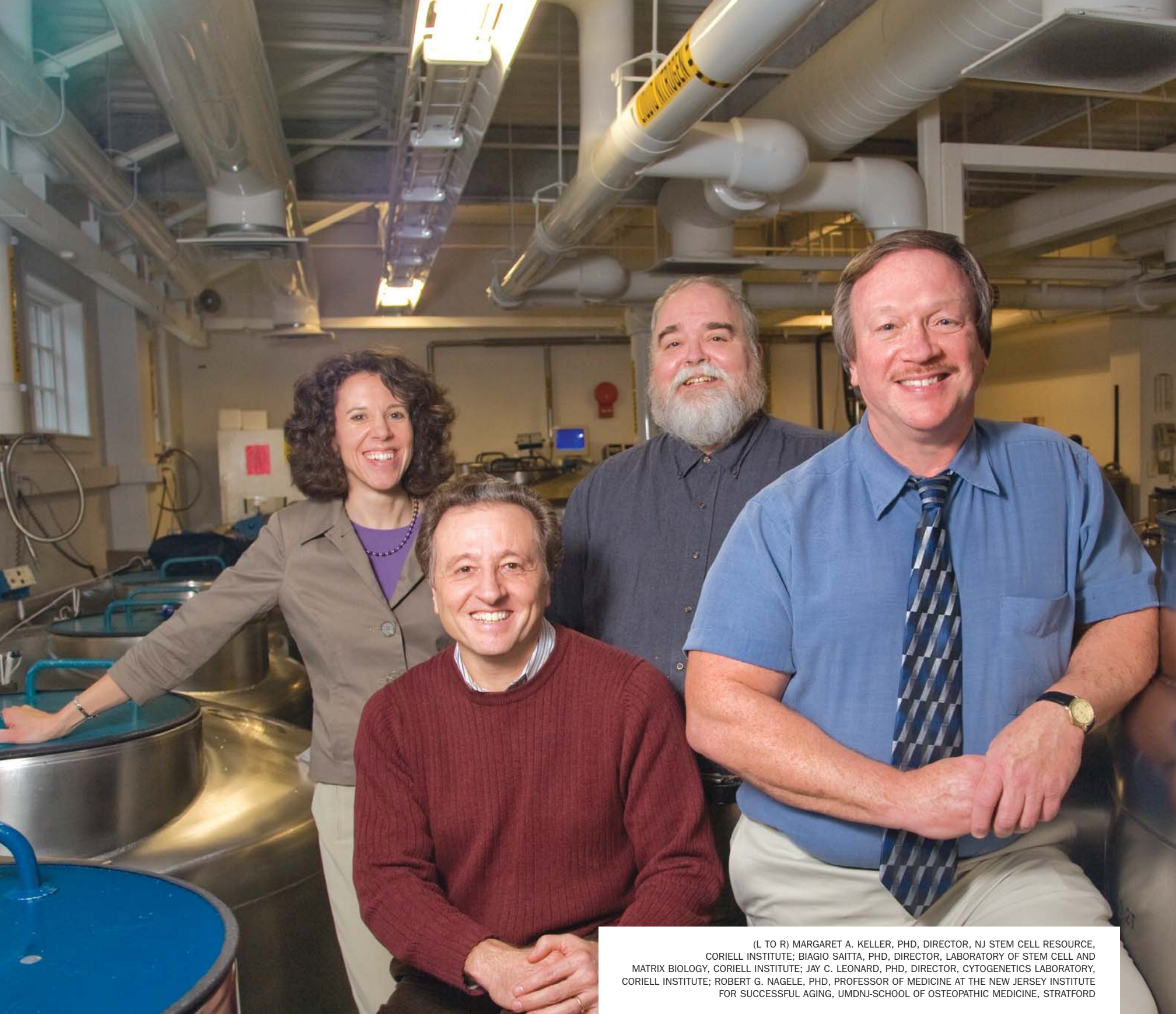
OUR GOAL IS TO EVALUATE THE GENOMIC STABILITY AND DIFFERENTIATION POTENTIAL OF MSCs DURING AND AFTER THEIR PREPARATION FOR CLINICAL USE — IN OTHER WORDS, TO MAKE SURE THAT TREATMENT OF PATIENTS WITH MSCs IS SAFE.

that are sufficient for clinical use. Unfortunately, by the time sufficient numbers of MSCs have been generated, there is a danger that the cell population may contain a significant number of cells that are no longer able to proliferate (a state of cellular senescence), as well as some genomically unstable cells that have somehow managed to bypass senescence. Collaborative studies being carried out in our laboratories at the NJ Institute for Successful Aging in Stratford and the Coriell Institute in Camden have focused on elucidating these potentially deleterious changes, with the aim

Our work on MSCs, which is currently funded by the New Jersey Commission on Science and Technology, is being done through a collaboration between my laboratory at UMDNJ in Stratford and those of Drs. Biaggio Saitta, Margaret Keller and Jay Leonard at the Coriell Institute. Our goal is to evaluate the genomic stability and differentiation potential of MSCs during and after their preparation for clinical use — in other words, to make sure that treatment of patients with MSCs is safe.

MSCs are extremely rare (only 1-3 per 10,000 cells). This requires

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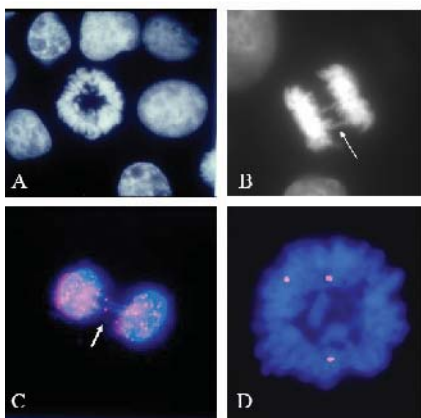


Figure 1.

A. Chromosomes are arranged into a ring-like (rosette) array at prometaphase. B. Chromosome end-to-end fusion causes chromosomes to stretch (arrow) and break during anaphase. C. Late anaphase showing chromosomes fused at their telomeric ends (pink dots and arrow) still bridging the gap between daughter nuclei. D. Prometaphase chromosome rosette of genomically abnormal (aneuploid) cell showing three copies of chromosome 8.



Figure 2.

An example of a spectral karyotype is arranged in the same form as the traditional G-band karyotype. It provides two views of each chromosome. The inverted DAPI (diaminophenylindole) chromosome image to the left provides a view of the overall chromosome morphology that is similar to G-banding. The classified color chromosome image to the right indicates the chromosome homology assigned by the spectral karyotyping program.

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them first to be isolated from surrounding cells and then coaxed to undergo extensive proliferation in a cell culture environment (*ex vivo* expansion) in order to generate numbers of these cells that are clinically useful. However, unlike ESCs, MSCs grown in culture undergo “*in vitro* aging” — they are subject to the Hayflick limit of roughly 50 mitotic divisions and then lose their ability to replicate further, achieving a quiescent state referred to as cell senescence. Since a major trigger that drives cells into senescence is DNA damage, this suggests that MSCs in long-term cell cultures are becoming progressively more genomically unstable — of course, an undesirable feature for cells that we intend to inject into patients. One type of DNA damage that potently induces senescence is the aging-dependent shortening of telomeres. Telomeres are long stretches of DNA that cap the free ends of chromosomes and are now thought to serve as the “cell aging clock.” They shorten with each mitotic division — making our telomeres progressively shorter as we age. Eventually, one or more telomeres become critically short and dysfunctional, and these are recognized as DNA damage. Attempts to repair such damage can lead to chromosome end-to-end fusions, which disrupt chromosome segregation during subsequent mitoses and cause genomic instability, including chromosome

breakage and numerical aberrations (aneuploidy) that are potentially tumorigenic.

Unfortunately, there is a danger that by the time sufficient numbers of MSCs have been generated for infusion into patients, the cell population may contain a significant number of senescent cells as well as some genomically unstable cells that have somehow managed to bypass senescence. Although the fate of these cells after injection into patients is unknown, a recent study has shown that human MSCs derived from adipose tissue can undergo spontaneous transformation after long-term culture and may be prone to malignant transformation. This same phenomenon has been observed in mouse MSCs. These findings emphasize the need for accurate studies that will document the bio-safety of these cells before their infusion into patients, especially in immune-compromised patients whose status might favor the development of tumors.

The goal of our collaborative study is to gain a thorough understanding of how *ex vivo* expansion of MSCs can affect their stemness (i.e., ability to act as stem cells), genomic stability, and proliferation and differentiation capacity. We are evaluating the genomic stability of MSCs at selected intervals during *ex vivo* expansion. This evaluation includes identification of abnormal and senescent cells and determination of chromosomal abnormalities in MSCs by G-banding, fluorescence *in situ* hybridization using chromosome-specific paint probes and spectral karyotyping (SKY). In addition, the effects of prolonged culture on telomere length at the single cell and individual telomere level in MSCs will be determined by quantitative image analysis. We are also investigating the effects of *ex vivo* expansion of MSCs on their chromatin organization. We predict an enrichment of acetylated histone H3 (acH3) on the proximal promoter regions of genes involved in maintaining the stem cells in their undifferentiated state (called “stemness genes”). We are testing whether MSCs at later culture passages show a reduction in the number of stemness gene promoters that show acH3 enrichment, and are also checking to see if these epigenetic changes coincide with alterations in the differentiation potential of MSCs and expression of their stemness genes. Lastly, it is essential to make sure that MSCs, after completing extensive growth in cell cultures, still retain their proliferation and differentiation capacity. To this end, we are measuring the ability of single MSCs to proliferate and form cell colonies and testing their ability to differentiate toward adipogenic, osteogenic and chondrogenic lineages. Our goal is to shed some light on genetic and phenotypic changes that occur in MSCs during their *ex vivo* expansion. Once any undesirable changes are identified, it may be possible to sidestep these changes by altering the *ex vivo* expansion procedures, thus further ensuring the safety and effectiveness of MSCs for therapeutic use.

Robert Nagele is a professor in the Department of Medicine and New Jersey Institute for Successful Aging at the UMDNJ -School of Osteopathic Medicine (SOM). He received his PhD in 1980 from Rutgers University and did his post-doctoral training at Rutgers Medical School. He joined the faculty at SOM in 1983. His research interests are focused on the mechanisms of aging-associated diseases, especially Alzheimer's disease and cancer. His work in these areas was funded by the Alzheimer's Association, The Governor's Council on Autism, the Foundation of UMDNJ and the New Jersey Commission on Science and Technology.

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Tumor stem cells in lung cancer

by JOHN LANGENFELD

Recent studies have linked the growth of cancer to a specific population of cancer cells that reside within a tumor. These specialized cells can form tumors on repeated injections into nude mice while the remaining cancer cells do not form tumors. The behavior of these specialized tumor cells resembles that of stem cells, so they have been named “tumor stem cells.” This data provides intriguing new insight into how tumors grow and will likely lead to novel treatment modalities. Present day chemotherapy is designed to treat the whole tumor. Future tumor therapy may be designed to treat only the “tumor stem cells.”

Many fundamental questions need to be answered before progress can be made for therapies targeted against “tumor stem cells.” It is clear that some tumor cells can initiate tumor growth while others do not. It is not clear if the tumor-initiating cell population represents one pure population or whether more than one cell population is present. We do not know whether cancers

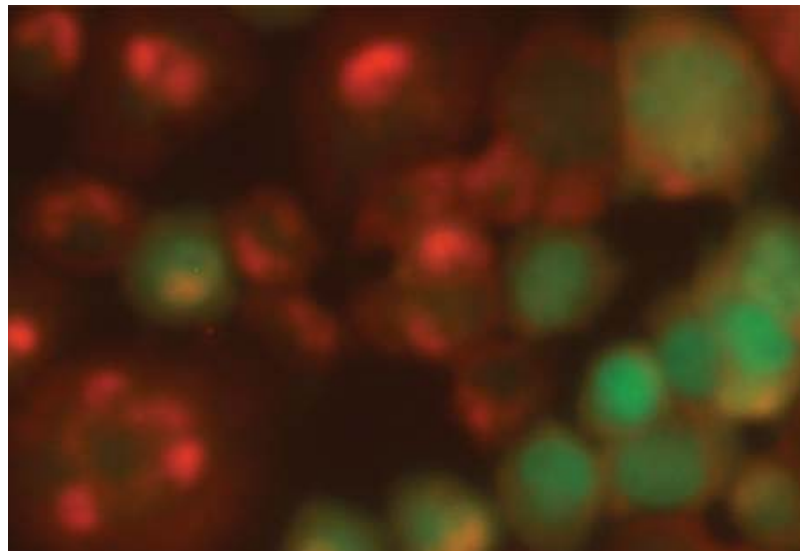


Figure 1.

By FACS, GFP expressing cells from the Oct4/GFP cell line were sorted and plated at a single cell density on glass cover slips. We then examined whether the Oct4/GFP cells differentiated into a neural lineage. (A) Colonies were immunostained for the neural marker NeuN. The NeuN expressing cells are red and GFP cells are green. Note: some cells lost GFP expression and became NeuN positive.

are composed of distinct lineages with different biological activity. It is also not clear whether more than one population of cells can initiate tumor growth. Another important question is whether “tumor stem cells” differentiate and, if so, what cell types do they differentiate into. There is evidence that in brain carcinomas, neural precursors differentiate. However, in other solid tumors, differentiation needs to be further elucidated.

In my laboratory, we have been studying the “tumor stem cell” population in non-small cell lung carcinomas. We found that surface markers used to identify tumor stem cells in other solid tumors were not helpful in isolating a homogenous cell population. We have developed, in lung cancer cell lines, a new method to isolate and study “tumor stem cells.” Our model utilizes the activity of the embryonic transcription factor Oct4 that is only

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