

ADD. DELETE. SWAP.



Stem Cell Society

Bridge the division of Social Equity through Scientific Discovery Knowledge Connect Us All

Session 1: Modulation of Gene Expression Using RNA Interference and Zinc Finger Nucleases

By Supriya Shivakumar, PhD, Sigma-Aldrich Corporation

Technologies that allow researchers to routinely modulate and efficiently edit the genomes of stem cells from virtually any species would greatly enhance the understanding of basic stem cell biology, and potentially lead to novel ways of treating human disease. The most common methodologies that alter gene expression do so at the RNA level in the form of RNA interference studies. Lentiviral delivery of shRNAs has been shown to be advantageous for difficult to transfect cell types, including both embryonic stem cells. We will discuss a double transduction protocol optimized to infect and knockdown targeted genes in embryonic stem cells. This protocol was used to knockdown expression of RhoA in feeder-free human embryonic stem cells, which resulted in differentiation into a neural precursor phenotype.

Rational genome engineering in mammalian cells allows for the study of heritable changes in gene expression. We discuss today a novel technology that enables high-frequency genome editing via the application of designed zinc finger nucleases (ZFNs). Within these chimeric proteins the DNA binding specificity of the zinc finger protein determines the site of nuclease action. Such engineered ZFNs are able to recognize and bind to a specified locus and evoke a double-strand break (DSB) in the targeted DNA with high efficiency and base-pair precision. The cell then employs the natural DNA repair processes of either homology-directed repair (HDR) or non-homologous end joining (NHEJ) to heal the targeted break. These two pathways provide the investigator with the ability to provoke three unique outcomes in genome editing – gene correction, gene deletion and targeted gene addition. Furthermore, the speed and efficiency of this process enables us to knockout multiple genes in the same cell. Drawing from our work with transformed cell lines, primary human cells, and multi-potent stem cells, we will present several examples of single, double and triple gene knockout in mammalian cells and knockouts of native genes in animals including rat. Furthermore, we demonstrate the use of ZFN technology for targeted gene insertion into native chromosomal loci in cells including human and mouse embryonic stem cells.

Session 2: Tools for Culturing Stem Cells

By Sheng Lan Cao, PhD, Sigma-Aldrich

Stem cells are characterized by their ability to either self renew through mitotic cell division or to differentiate into a diverse range of specialized cell types. Adult stem cells function as a natural repair system for the body by replenishing specialized cells, and maintaining the normal turnover of regenerative organs, such as blood, skin or intestinal tissues. Highly plastic adult stem cells are derived from umbilical cord blood and bone marrow, and are routinely used in medical therapies and stem cell research. Stem cell research is an exciting field that promises fantastic curative discoveries in numerous areas from cancer to diabetes to neurodegenerative diseases. There will be a discussion on the tools used for the culturing of neural stem cells, hematopoietic stem cells, mesenchymal stem cells and 3D Cell cultures such as MaxGel™ human Extracellular Matrix (ECM), HydroMatrix™ synthetic peptide, and mouse ECM. 3D environments provide cells a better ability to mimic their in vivo counterparts. There will also be data presentation on the culturing of neuronal stem cells and the use of 3D culturing systems to generate viable differentiated neuronal cells for the treatment of traumatic brain injury in a rat model.

Limited seats! Register online for this free seminar!

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