17th Stem Cell Club Meeting

(Organised by the Stem Cells Research Singapore Website Committee http://www.stemcell.edu.sg)

Date: November, 15th 2006 (Wednesday)
Time: 5:00 pm
Venue: Matrix, 4th Level, Breakthrough Theatrette

Host: Gerald Udolph, CMM

Time	Title	Speakers
5:00-5:40	***Designing and Validation of successful RNAi Asssays***	**Caine Leong** *Biorad Singapore*
5:40-6:20	***Characterization of ABC-transporters, EGF, FGF, and Neurotrophin signaling in neural stem/progenitor cells***	**Mohammed Islam** *NTU*
6:20-7:00	***From chromatin structure to the maintenance of pluripotency in hES cells***	**Peter Droge** *NTU*
7:00-	**Networking with cheese and wine**	

This event is sponsored by BIO-RAD

Designing and Validation of Successful RNAi Assays

Caine Leong
Product Specialist, Bio-Rad Laboratories (Singapore) Pte Ltd

RNA interference (RNAi) is a phenomenon where dsRNA specifically blocks the expression of its homologous gene. The advent of RNAi mediated 'knockdown' with specific siRNA molecules allows for targeted, inexpensive and rapid analysis of gene function in mammals. In nature, longer dsRNAs associate with Dicer endonuclease, a member of the RNase III family, which precisely cleaves the dsRNA into smaller functional siRNAs. These siRNAs then associate with an RNA-induced silencing complex (RISC), which targets any homologous mRNA for degradation. It has recently been suggested that in addition to cleaving longer dsRNAs, the Dicer also plays a role in loading processed dsRNA into the RISC complex. This hypothesis led to the development of a new class of siRNAs, termed **Dicer-substrate siRNAs (siLentMer)** that are even more powerful effectors of gene-specific silencing than 21-mers. In addition, these siLentMer siRNA duplexes can also mediate effective silencing at extremely low concentrations **(≥ 85 % knockdown using 5 nM of siLentMer)**. With the aim of illustrating the general workflow of RNAi, we used lipid-mediated delivery of a pre-designed siLentMer™ for beta-actin to discuss the design, delivery and validation of RNAi-based assays.

Characterization of ABC-transporters, EGF, FGF and Neurotrophin signaling in neural stem/progenitor cells

Mohammed Omedul Islam and Klaus Heese
School of Biological Sciences, Nanyang Technological University, Singapore

Neuronal survival is a major concern in the aging brain and neurodegenerative diseases of the central nervous system (CNS). In recent years it has been noted that the adult brain has the self-repair capacity to replace lost neurons in several select regions of the CNS, such as the olfactory bulb, the hippocampus, the adult human subependymal zone and the cortex. Estimations of the number and proliferative capacity of these cells suggests the possibility that therapy through replacement of lost cells by mobilizing endogenous stem cells already present in the brain or transplantation of exogenous cells may be a realistic therapeutic approach. The question facing modern medicine is how best to use neural stem cells (NSCs) to produce functional recovery in neurodegenerative disorders in the aging brain. Neural transplantation of NSCs is applied to clinical trials for many degenerative neurological diseases, including Parkinson's, Alzheimer's (AD), Huntington's diseases and stroke. Multipotent NSCs, capable of giving rise to neurons, glia and oligodendrocytes, line the cerebral ventricles of all adult brains. A number of approaches have been evolved for using neural progenitor cells in cell therapy. NSC-grafts and the stimulation of endogenous NSCs represent potentially alternative therapeutic approaches to replace lost or damaged neurons in AD, but the major challenge of stem cell transplantation is how to isolate pure NSCs form heterogeneous neurospheres to generate a specific neuronal subtype and a sufficient number of target neurons (such as cholinergic neurons for the treatment of AD patients) after transplantation. In the present lecture I will present recent data about: (1) the isolation and characterization of human neural stem/progenitor cells from human fetus brain; (2) the effect of EGF and FGF on their proliferation, phenotype marker expression and differentiation; (3) the differentiation capacity of neurospheres into specific neuronal subpopulations, and (4) the separation and characterization of neural stem/progenitor cells from neurospheres using a specific ABC transporter protein (MDR1) antibody. In addition, I will discuss preliminary data showing the involvement of neurotrophins (NGF, proNGF, BDNF, NT-3, NT-4/5) and their specific receptors (TrkA, TrkB, TrkC and p75NTR) in NSCs signaling events during neural stem/progenitor cells survival and differentiation.

From chromatin structure to the maintenance of pluripotency in hES cells

Peter Droge
School of Biological Sciences, Nanyang Technological University, Singapore

The state of chromatin in human embryonic stem (hES) cells is a key factor determining stem cell identity. The non-histone chromatin-associated factor HMGA2 has been studied mostly in the mouse where its function seems critical for embryonic cell growth and adipocytic cell differentiation. In the first part of the presentation, we show that HMGA2 is highly expressed in two undifferentiated human embryonic stem cell lines at a level of at least 10^5 copies per individual stem cell. Interestingly, expression is further up-regulated by a factor of three at day 7 of embryoid body formation, before it quickly drops to or below the level found in undifferentiated cells. We also show that HMGA2 is stably associated with inter- and metaphase hES cell chromatin, and that up to 12 HMGA2 protomers stably associate *in vitro* with a single nucleosome core particle of known atomic structure. Our data lend support to the possibility that HMGA2 interacts with nucleosomes in a way that imposes a global effect on the state of ES cell chromatin which may contribute to the establishment of both ES cell identity and the initiation of specific differentiation programs. Experiments are under way to probe this directly using siRNA and microarray technology.

In the second part of the talk, I present a *UTF1*-based transgenic system for the maintenance of undifferentiated hESCs. Transcription of the pluripotency marker gene *UTF1* in hESCs ceases at the onset of differentiation. We show that two transgenic hESC lines bearing *UTF1* genetic elements that drive a resistance gene exhibit a more robust phenotype towards spontaneous and directed differentiation, and that antibiotic selection leads to homogeneous undifferentiated hESC cultures. Hence, our transgenic system could be a valuable tool for many hESC applications and cell reprogramming strategies.