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A4/C3–STEM CELLS AND REPROGRAMMING

Organised by Stefan Przyborski and John Bryant for the Cell Biology Section

A4/C3.1 Abstract not supplied

A4/C3.2 Stem cells, progenitors and myelin repair

Robin JM Franklin, University of Cambridge

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, provides one of the best examples in neurobiology of a multipotent progenitor cell contributing to regeneration in the adult CNS. Oligodendrocyte progenitor cells (OPC) in normal adult white matter constitute a stable population of quiescent cells that divide infrequently. However, following oligodendrocyte loss and demyelination, such as occurs in multiple sclerosis, OPCs respond by proliferation and migration and finally differentiation into myelin sheath forming oligodendrocytes. This presentation will focus on three issues: first, the environmental factors that orchestrate this process, second, the intrinsic changes that occur within activated OPCs, and third, the heterogeneity of the OPCs population and its relationship to neural stem cells and other neural progenitor cells.

A4/C3.3 Abstract not supplied

A4/C3.4 Abstract not supplied

A4/C3.5 Abstract not supplied

A4/C3.6 Abstract not supplied

A4/C3.7 Embryonal carcinoma cells as embryonic stem cells and their capacity for neural differentiation

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Embryonal carcinoma (EC) cells are the stem cells of germ cell tumours and are considered the malignant counterparts of embryonic stem (ES) cells. While it should always be remembered that EC cells possess an abnormal karyotype, they remain a useful model to study certain aspects of cellular differentiation. In particular, human TERA2-derived EC stem cells prove to

be valuable tools to study neural development in a manner pertinent to human embryogenesis. The EC line, TERA2.cl.SP12, was derived directly from the earliest available passage of the primary tumour explant culture, TERA2, using a combination of immunomagnetic selection and single cell cloning. Under specific growth conditions, TERA2.cl.SP12 cells provide an excellent model system to study cell fate determination and the specification of neural and epidermal lineages (for example, >95% can form neural derivatives). Using various molecular approaches including micro-array analysis, we have shown that neural differentiation by human TERA2.cl.SP12 cells is conserved and results in functional neuronal cell types. In some ways, the capacity of EC cell differentiation is limited, whereas ES cells will extend the range of questions that can be addressed *in vitro*. On the other hand, the limitation of EC cells can sometimes be put to advantage, as they provide a simpler and more experimental system. Human EC and ES cells are likely to remain complementary tools for research and development.

A4/C3.8 Neural differentiation of mammalian bone marrow stromal cells

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Mammalian bone marrow stromal cells (BMSCs) have the capacity to differentiate into several types of mesodermal tissues. In addition, recent research has demonstrated the ability of these cells to differentiate into non-mesodermal tissue types, including neural like cells. In our laboratory we have developed protocols for the neural differentiation of BMSCs under culture conditions previously documented, including exposure to antioxidants and conditions known to induce neural differentiation of embryonic stem cells, including retinoic acid (RA), nerve growth factor (NGF) or brain derived neurotrophic factor (BDNF). Western blot analysis, flow cytometry and immunofluorescence detection has demonstrated the expression of intracellular and cell surface markers associated with a neural phenotype.

There were significant differences in both the percentage and phenotype of the neural-like cells generated from these protocols. Neural induction using antioxidants results in >90% generation of neural-like cells in contrast to RA + BDNF/NGF in which 10–15% of cells respond. In addition, exposure to antioxidants resulted in the production of neural cells only with the absence of GFAP expression that is detected at low levels following BDNF treatment. We hypothesise that these differences may arise due to the heterogeneity of the BMSC culture. There is however, little known about the heterogeneity of BMSC populations *in vitro*. We are currently examining which subpopulations of cells respond to the various induction agents.

A4/C3.9 Embryonic stem cells and telomerase

Majlinda Lako

Embryonic stem cells are derived from the inner cell mass of blastocysts and can be maintained indefinitely in culture under appropriate conditions. They are characterised by the expression of several transcription factors such as Oct4, Nanog, cell surface markers such as stage specific embryonic antigens (SSEA) and high activity of alkaline phosphatase and telomerase enzymes. Telomerase is a ribonucleoprotein complex that helps to maintain telomeres structure and length. In most human cells that lack telomerase activity for most of their cell cycle, chromosome ends shorten at each cell division, limiting the replicative lifespan and leading eventually to senescence. The presence of telomerase in embryonic stem cells has been linked to their indefinite replication in culture. We and others have shown that telomerase activity and the expression of the main component of the telomerase, (Tert) is reduced during the differentiation of embryonic stem cells. We modulated

the expression of Tert and telomerase in embryonic stem cells and investigated the effects of this regulation on the embryonic stem cells and their ability to differentiate towards haematopoietic lineages. We found that overexpression of Tert caused enhanced cell proliferation, whilst its downregulation led to slower cell growth and accumulation of cells into G0/G1 phase of the cell cycle. In addition, downregulation of Tert led to shortening of telomeres, accumulation of chromosomal aberrations in ES cells and reduced ability to differentiate towards haematopoietic lineages. Overexpression of Tert rendered ES cells more resistant to apoptosis and oxidative stress and more efficient at generating haematopoietic cells. Our preliminary results suggest that this effect is due to enhanced proliferation of haematopoietic progenitor cells rather than enhanced induction of haematopoietic stem cells. Microarray studies have indicated that a number of genes involved in protection from oxidative stress such as Bmi, Gpx2, Gsta3, Hspa1a are decreased during the differentiation of ES cells which implies that the majority of differentiated progeny cells have a somewhat reduced requirement to remove reactive oxygen species a possibility which is mirrored by the observed accumulation of peroxide in the cells of EBs as they progress through differentiation. Murine ES cells that have higher Tert expression do retain the expression of these genes during differentiation and their ability to remove reactive oxygen species efficiently remains intact.

Recently we have been successful in generating a new human embryonic stem cell line, hES-NCL1. It will be important for therapeutic reasons to investigate whether overexpression of TERT in human ES cells will lead to production of differentiated progeny which is less prone to replicative stress, more resistant to apoptosis and better at engrafting in the host tissue.

A4/C3.10 Abstract not supplied