WA15

Stem cells for signalling and disease mechanism studies

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Stem cells are believed to provide a tool by which new cells and tissues can be made and by which damaged ones can be replaced or repaired. Besides the enormous impact in the cell replacement field, and in particular for the central nervous system (CNS) since cell transplantation might help to overcome the intrinsic poor capability of the nervous tissue to replace elements lost in the course of injury or disease, the stem cell technology is becoming particularly important for others applications. Indeed, with a recent growing awareness of how stem cells can be made to grow in an unlimited, but regulated manner and how their fate can be directed or manipulated into mature phenotypes in culture, it has become clear that the biological resource offers additional attractive features applicable for future biomedical research. While it is also anticipated that stem cells of human origin will provide reagents that will revolutionise aspects of biomedical and pharmacological fields, in the last few years, stem cells have been proved a valuable tool for modelling diseases and studying signalling mechanisms in order to obtain optimized drugs. Here, data will be presented about the employment of ES cells and Neural Stem cells for:
1) the investigation of signalling commitments from the Shc family of signal transduction molecules during the induction, the proliferation and neuronal differentiation events in the developing brain
2) the generation of optimized cellular systems for the study of the molecular mechanisms underlying neurologic disease, and in particular Huntington's disease

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

WA16

Use of adult stem cells to treat brain and spinal cord injury

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During the last decade, medical research has made immense progress in conquering many devastating diseases. Current neurotransplantation research focuses on the potential of neural grafts to replace damaged cell populations, the production of missing transmitters and neuroactive substances as well as on the delivery of growth factors such as BDNF, GDNF or NGF. The limited regenerative capacity of the adult central nervous system (CNS) remains a major stumbling block in the development of effective therapies for neurodegenerative diseases such as Parkinson's disease, multiple sclerosis, Alzheimer's disease or brain and spinal cord injury. Neural as well as non-neural stem cell (SC) therapy might overcome the low regenerative capacity in the human CNS.

Embryonal stem cells (ESC) and bone marrow stromal cells (MSC) are pluripotent progenitor cells that have the capacity to migrate towards lesions and induce or facilitate site-dependent differentiation in response to environmental signals. In our studies, we examined the behavior of mouse ESC and rat or human MSC grafted to injured rat brain and spinal cord. We studied whether these cells are capable of survival and if they participate in lesion repair, differentiate into neurons and astrocytes, prevent scar formation or promote neurogenesis.
The migration and fate of ESC and MSC were studied using cells labeled in culture with magnetic iron-oxide nanoparticles. The cells were transplanted into adult rats with a cortical photochemical lesion or a balloon-induced spinal cord lesion. In vivo MR imaging was used to track cells; electron microscopy and Prussian blue staining confirmed the presence of nanoparticles inside the cells. SC labeled with nanoparticles preferentially migrated into the lesion. In the case of MSC, only a few of the cells that entered the lesion expressed the neuronal marker NeuN and even fewer GFAP-positive cells were detected. There was no significant difference in the number of MSC entering the lesion between animals directly injected in the cortex or spinal cord and those systemically infused. We found that the intravenous injection of MSC 24 hours after spinal cord injury improved the behavioral outcome of the animals (BBB score and plantar test) starting at 4 weeks after implantation, presumably by the production of regeneration-promoting factors as yet unknown.
The implantation of biocompatible polymer hydrogels can reduce scar tissue formation and bridge a lesion, providing a scaffold to reform the tissue structure. We implanted blocks of biodegradable hydrogels based on copolymers of 2-hydroxypropylmethacrylamide with ethoxyethylmethacrylate into rats with hemisected spinal cords. We used hydrogels with in vitro degradation times of 7, 13 or 35 days as well as control nondegradable hydrogels. The animals were sacrificed 28 days after implantation. Both the nonbiodegradable and biodegradable hydrogels were biocompatible and adhered well to the host tissue, bridging the whole spinal cord lesion; with the nondegradable hydrogels, tissue adhesion was less pronounced. All biodegradable hydrogels degraded from the border that was in direct contact with the spinal cord tissue. They were resorbed by macrophages and replaced by newly formed tissue containing connective tissue elements, blood vessels, astrocytic processes and neurofilaments. These gels were further used as 3D carriers for MSC and implanted into rats with spinal cord injury. Our study shows that biodegradable polymer hydrogels are promising candidates for bridging gaps in the central nervous tissue. These hydrogels are in many ways similar to the environment in developing nervous tissue and can mechanically support ingrowing cells and axons. Their chemical and physical properties can be tailored to a specific use, and in the future they may be used in human medicine as 3D carriers for stem cell implantation.
Our clinical study in patients with transversal spinal cord lesions is based on the above experimental results. MSC implantation is being used in a Phase I clinical trial in patients with transversal spinal cord lesions (n=20). We compared intra-arterial vs. intravenous administration and a group of acute (10-30 days post-injury) vs. chronic patients. We can conclude that implantation is safe, as there were no complications following MSC

administration. We are using MEP, SEP, MRI and the ASIA score in our patient follow-up. Although improvement of motor and sensory functions has been observed below the lesion site, further trials involving a much larger population of patients are needed before any conclusions can be drawn.

These studies demonstrate the immense potential of SC as a therapeutic tool in the treatment of injury and degenerative diseases. It is evident that there may be various ways in which SC may interact with the host CNS tissue.

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WA17

The Use of Cells from the Olfactory System for CNS Repair

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The peripheral olfactory system exhibits a remarkable regenerative capacity; a capacity which is demonstrated both by the continuous neurogenesis of olfactory receptor neurons in the olfactory epithelium and by the fact that new olfactory axons are able to grow into the adult CNS environment of the olfactory bulb. It is thought that these properties are in part due to basal stem cells in the olfactory epithelium that generate new olfactory receptor neurons, but also specialised glial cells that reside in the olfactory mucosa and bulb, termed olfactory ensheathing cells (OECs) which direct the newly generated axons to their target in the olfactory bulb (Barnett and Chang, 2004). We will describe the differentiation capacity of olfactory neural cells in culture from the olfactory mucosa and olfactory bulb. We will demonstrate that cells from the horizontal basal layer in the olfactory mucosa are able to differentiate into OECs. In addition we will describe the specific properties of OECs that make them a promising candidate for the transplant-mediated repair of CNS lesions (Fairless et al., 2005). A detailed comparative study has been made between OECs and the glial cell from the peripheral nervous system, Schwann cells. In these studies we find that OECs but not Schwann cells are able to mingle with astrocytes to a greater extent and do not induced in them characteristics typical of hypertrophy. Lastly, experiments will be described in which green fluorescent protein (GFP) labelled olfactory cells are transplanted into a model of spinal cord injury. In this model the ascending branches of the primary afferent fibres into the dorsal columns are cut using a wire knife. Data will be presented to show how the transplanted cell survive integrate and support regeneration of the dorsal columns.

Barnett SC and Chang L. (2004) Trends Neurosci. 27, 54-60.

Fairless R, et al.,. (2005) Molecular Cell. Neurosci. 28:53-263

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WA18

Epidermal stem cells: origin, phenotypic characterization and function

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Epidermis contains similar to other tissue types a pool of cells responsible for self-renewal of the epithelium and its regeneration. These cells, so-called epidermal stem cells (ESC), are characterized by a high proliferative potential. Transit amplifying cells, as daughter cells of ESC, proliferate rapidly, but their potential is restricted. ESC are located in the bulge region of the outer root sheath of hair follicle or in the basal layer of interfollicular epidermis. In addition to keratinocytes, ESC also yield cells of sebaceous glands and of hairs. Stemness of ESC and initiation of differentiation are guided predominantly by Shh and Wnt signaling cascades. No specific markers of ESC were so far discovered but it is known that these cells highly express integrin receptors and keratin 15 and 19 are also present in these cells. Nuclear expression of p63 is also connected with ESC phenotype. Based on the emerging concept of of the sugar code study of endogenous lectins was initiated (Gabius et al., 2004). The growth regulatory endogenous lectin, galectin-1 and epitopes reactive for this lectin are expressed in nuclei of these cells. As indication for specificity, they are never bound by another endogenous lectin, galectin-3 (Purkrabkova et al., 2004). The establishment of squamous cell carcinoma appears to be connected with mutation of ESC or progenitor elements. Application of ESC in advanced cell therapy of skin defects and use the development of new treatment strategies of carcinomas arising from insights into mechanisms of differentiation in the treatment of carcinomas represent the perspectives for further research in this area.

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