1050. Adenoviral Clostridial Light Chain Expression in the Medial Forebrain Bundle: A Reversible Model of Dopamine Depletion

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Here we demonstrate that cells transduced with SFß91 XRCC1 IRES eGFP acquire a 6 fold increased resistance to camptothecin and 5-iodo-2'-deoxyuridine and display increased kinetics of repair. Resistance to these agents could be reversed by addition of either the PARP inhibitor 3-aminobenzamide, or methoxyamine (an inhibitor of base excision repair) respectively. Furthermore, cells transduced with XRCC1 demonstrate a significant increase in protection against ionising radiation. In summary, retrovirally-mediated transfer and expression XRCC1 to cells of the primary haemopoietic compartment delivers both physical and biological protection against the action of agents that cause either direct or indirect DNA strand breaks. Our findings are encouraging for the development XRCC1 gene transfer for protection against the myelotoxic side effects of chemotherapy using camptothecin or its derivatives and we are currently evaluating this therapy in *in vivo* toxicity models.

1048. Engineering and Development of I-AniI Homing Endonucleases for Gene Correction Applications

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Induction of a double strand break in DNA results in a large increase in the frequency of homologous recombination in the vicinity of the double strand break, raising the possibility of targeted gene correction if reagents were available for the simultaneous introduction of a site specific double strand break and a correcting DNA fragment. Structural and molecular studies of homing endonucleases (HE's) suggest that HE's possess significant potential for engineering of their cut site specificities to diverse target sequences. Here we discuss preliminary work on the I-AniI endonuclease with regards to its application to targeted gene correction of primary immune deficiencies. Work to be discussed will focus on the engineering hurdles to be faced in applying I-AniI as a site specific DSB-inducing tool, and the development of model systems for the evaluation and benchmarking of HE's and other DSB-inducing reagents in practical gene correction methods.

1049. Serum Free Fedia Is the Best for Cord Blood Hematopoietic Cells Expansion

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Objectives: to find the best cell culture condition for expansion of cord bloods hematopoietic CD34+/CD38- ex vivo expansion and study the role of MIP/a in expansion potential.

Material and methods: CD34+/CD38- cells separated from cord bloods by Mini-MACS and cultured in different culture mediums including 50ng/ml of TPO,IL-6,SCF and flt3-ligand.for some samples we used RPMI+ 10% FCS or autologous cord blood plasma and for others we used serum free media(SF). Also we added MIP/a (10-50ng/ml) to some samples. We cultured the cells for 2 weeks in CO2 incubator and studied expansion potential by counting of MNCs ,CFU-assay , LTC-IC and studied the number of CD34+/CD38- cells before expansion 7 days and 14 days after expansion.

Results: expansion potential of cord blood hematopoietic cells was good and maximally expanded to 25 times. Also we found that serum free media isbetter than FCS 10% and autologous cord blood plasma. MIP/a did not changed expansion potential of hematopoietic cells.

Conclusion: serum free media is the best medium for expansion and from GMP points of view and MIP/a is useful for expansion to prevent maturation during expansion that may be useful for increasing transduction efficiency without induction of maturation.

GENE REGULATION: REGULATED SYSTEMS AND TISSUE SPECIFIC EXPRESSION

1050. Adenoviral Clostridial Light Chain Expression in the Medial Forebrain Bundle: A Reversible Model of Dopamine Depletion

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We have previously demonstrated focal synaptic inhibition through neuronal expression of the light chain (LC) fragment of tetanus toxin *in vivo*. The transient effects are spatially discrete lending them to application in deep brain nuclei. This experiment examines feasibility in creating a rat model for Parkinsons disease through gene-based synaptic inhibition of the substantia nigra. The present experiment examined the impact of nigral LC expression on apomorphine induced rotations.

Methods: Tetanus light chain (LC) was cloned into an adenoviral vector under control of the CMV promoter containing a GFP marker. Next, the impact of unilateral nigral 6-OHDA on striatal dopamine and glutamate synapses was compared to unilateral nigral LC expression. Rats received medial forebrain bundle (MFB) injections of either 4 or 8µL of AdTeTxLC, 4µL of 6-OHDA or PBS. Apomorphine-induced rotational behavior was assessed using a rotometer weekly for up to 4 weeks.

Results: A significant increase in contralateral rotation was observed in the 6-OHDA positive control group and the 8µL TeTxLC group, in comparison to the 4µL TeTxLC and PBS groups. 6-OHDA animals demonstrated an average of 7.84 rotations per minute (+/- 0.45 SEM) and rats receiving 8µL TeTxLC demonstrated an average of 4.39 rotations per minute (+/- 0.41 SEM). PBS rats demonstrated an average of 0.325 rotations per minute and rats receiving 4µL TeTxLC demonstrated an average of 0.708 rotations per minute.

Significance: This initial model proves the feasibility of dopamine depletion through nigral LC expression. Because LC expression inhibits synaptic activity without killing neurons, this approach represents a strategy for transient dopamine depletion. A subsequent experiment will apply an adeno-associated vector containing a Tet-on expression cassette (rAAV.Tet-on.LC). This latter vector will facilitate controlled, transient nigral suppression and will facilitate the study of behavioral recovery and normalization of striatal receptors following the recovery of striatal dopaminergic input. Transient and controlled nigral inhibition may provide a superior model for studying striatal recovery and dopaminergic re-innervation.