Nonviral Gene Therapy for Bladder Cancer: Gene Delivery to Malignant Human Cells In Vitro and Ex Vivo

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Aim: Gene transfer-based therapies are being investigated as a new modality for the treatment of superficial bladder cancer. Having developed a novel, efficient and reproducible system of non-viral liposomal transfection (dioleoyltrimethylaminopropane (DOTAP) and methyl-β-cyclodextrin (MBC) solubilised cholesterol) of murine urothelial cells, we aim to determine its efficacy and efficiency for gene delivery to human bladder cancer cell lines in vitro, and to human normal and bladder cancer tissue specimens ex vivo.

Methods: Human bladder cancer cell lines (MGH, RT4) were transfected in vitro with our transfection system carrying the Beta-galactosidase marker gene (p-cmv-beta-gal). Cells were stained with X-gal reagent as reporter for Beta-galactosidase activity. Fresh Transitional cell carcinoma (TCC) and normal urothelial specimens were obtained using "cold-cup" biopsies, from the bladders of 20 patients during transurethral resection of bladder tumour (TURBT) for superficial bladder cancer. Harvested tissues were immediately placed in "Transport Medium" (RPMI complete medium) at 4°C, processed and disaggregated within 24 hours of resection. The tissues were similarly transfected ex vivo, incubated overnight and stained with X-gal reagent. Specimens were embedded in paraffin wax, sectioned and examined under light microscopy.

Results: Most human bladder cancer cell lines demonstrated transfections at high efficiency. Human urothelial and bladder cancer explants were also successfully transfected, mainly into the superficial epithelial layers.

Conclusion: We demonstrate for the first time that transfection of human urothelial and bladder cancer cells and tissue, using our novel newly developed non-viral liposomal transfection system is feasible and efficacious. The next step will be in vivo transfection of the human urinary bladder.

Endoscopic Ultrasonography-guided Fine-needle Aspiration (EUS-FNA) Immediately after Unrevealing Transbronchial Needle Aspiration in the Evaluation of Mediastinal Lymphadenopathy: A Prospective Study

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Aim: To determine the utility of transbronchial needle aspiration **(**TBNA) with rapid on-site cytology (ROSE) combined with the option for immediate endoscopic ultrasonography-guided fine-needle aspiration (EUS-FNA) in a one-session approach to mediastinal lymphadenopathy.

Methods: We prospectively recruited 20 patients with mediastinal lymphadenopathy on computed tomography who required cytologic evaluation. Bronchoscopy was first performed with TBNA and ROSE. If this was unrevealing, EUS-FNA was performed immediately afterwards. All procedures were done under local anesthesia and sedation with the same cytotechnologist in attendance.

Results: TBNA specimens were deemed adequate on-site in 13 patients and EUS-FNA was performed in the remaining 7. TBNA with ROSE was falsely negative in one patient. The diagnostic yield for TBNA and EUS-FNA alone was 70% and 86%, respectively. Combining the 2 provided a yield of 90% with no complications.

Conclusion: Performing EUS-FNA in the same session raised the diagnostic yield of TBNA alone from 70% to 90% and was safe and convenient. The cost-saving implications are obviating the need for patients to return for a second procedure and reducing the necessity for invasive mediastinal sampling with the higher yield.

Regional Selectivity of Medial Temporal Lobe Volumes in Schizophrenia

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Aim: Hippocampal and parahippocampal volume reductions are found in patients with schizophrenia but the relative contributions of these 4 regions (hippocampus, entorhinal, perirhinal and parahippocampal cortices) to medial temporal lobe pathology have never been examined. We tested the hypothesis that there are region selective reductions of these medial temporal lobe volumes in patients with schizophrenia.

Methods: We studied 19 male patients with schizophrenia and 19 age-matched male control subjects. Hippocampal and parahippocampal subregional volumes were estimated using a three-dimensional morphometric protocol for the analysis of high-resolution structural magnetic resonance images and repeated measures ANOVA was used to test for region-specific differences.

Results: Two main effects explained significant variance components of the medial temporal lobe volumes: diagnosis, i.e., healthy controls had greater volumes than patients [$F(1, 36) = 7.32$, $P = 0.01$] and region [$F(3,34) = 99.35$, $P < 0.001$]. The significant volume difference between the 2 groups was not specific for any of the regions [diagnosis by region interaction: $F(3,34) = 0.67$, $P = 0.58$] or for hemisphere [diagnosis by hemisphere interaction: $F(1,36) = 0.69$, $P = 0.41$ and diagnosis by hemisphere by region interaction: $F(3,34) = 2.65$, $P = 0.06$].

Conclusion: Previous studies of medial temporal lobe pathology have typically limited their search either to the hippocampus or the parahippocampal gyrus, leading to models of primarily hippocampal or parahippocampal pathology in schizophrenia. Medial temporal lobe reduction in the absence of regional selectivity has important implications for understanding the structural and functional integrity with which the parahippocampal region is related to the hippocampus in patients with schizophrenia.

Evaluation of Glenoid Labral Injuries with Non-Arthrographic Magnetic Resonance Imaging

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Aim: Direct magnetic resonance (MR) arthrography is highly sensitive and specific in detecting glenoid labral injuries. However, it requires the injection of contrast into the joint space, converting the usually non-invasive MR study into an invasive procedure. In this study, we sought to evaluate the adequacy of non-arthrographic MR imaging in the detection of glenoid labral injury by correlating them with

arthroscopic findings.

Methods: The sample population was drawn from 212 non-arthrographic shoulder MR examinations performed at our institution over a 19-month period. The MR imaging protocol consisted of axial, coronal oblique and sagittal oblique fast spin-echo proton-density weighted sequences. Patients underwent shoulder arthroscopy by the same surgeon within a year of imaging. A total of 33 shoulders were studied. Findings were taken to be concordant if the MR diagnoses correlated with the arthroscopic findings anatomically. The presence of associated Hill-Sachs lesions on MR was also studied.

Results: Using arthroscopy as the gold standard, the overall sensitivity and specificity of non-arthrographic MR imaging in detecting labral injuries are 91% and 64%. The sensitivity and specificity for detecting infero-anterior labral injuries are 89% and 93%. We also found that MR-diagnosed Hill-Sachs lesions have a 93% positive predictive value for true labral tears.

Conclusion: Non-arthrographic MR imaging of the shoulder is both sensitive and specific in detecting labral injuries associated with anterior glenohumeral instability, retaining the advantage of MR being a non-invasive study. Further experience and awareness of normal variants may help to improve the overall specificity of this examination.

Transplantation of Embryonic Cells with Endothelial Potential Immediately after Myocardial Infarction Lowers Mortality Rate and Reduces Myocardial Damage in the Murine Model of Acute Myocardial Infarction

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Aim: Our aim was to investigate the effect of the previously described RoSH endothelial potential progenitor cell line, in the treatment of the C57BL/6J murine model of acute myocardial infarction (AMI).

Methods: The left anterior descending (LAD) vessel was ligated and mice were injected intramyocardially with either 2 × 105 RoSH cells or an equal volume of NaCl solution at the apex of the heart. Mice were sacrificed and analysed in the immediate post-surgical period, 3, 10 and 21 days post-surgery. The measured parameters included: a) Post-surgical mortality rate; b) morphometric analysis of the absolute infarct size, ratio of infarcted to total left ventricular (LV) area and LV free wall thickness; c) grafting of transplanted cells in infarcted tissue by X-gal staining and PCR analysis for reporter genes; and d) cellular proliferation and vascular differentiation by assaying immunoreactivity for PCNA, a proliferation marker and PECAM (CD31), an endothelial-specific marker.

Results: Post-surgical mortality rate was significantly reduced. Absolute infarct size and ratio of infarcted to total left ventricle area were smaller compared to the sham treated hearts. RoSH cells were not present beyond 10 days post-transplantation. They were found only in areas of inflammation and in the endothelium of blood vessels. After 3 weeks, there was an increase in PCNA and PECAM staining per unit area in the cell transplanted compared to the sham treated hearts.

Conclusion: Thus, we show that transplantation of RoSH cells (embryo-derived cells with endothelial potential) post-induction of AMI is associated with a significant reduction in the absolute and relative infarct size.