54 AUTOLOGOUS MESENCHYMAL STEM CELLS AS CARRIERS OF ONCOLYTIC MEASLES VIRUS FOR CANCER THERAPY


Ovarian cancer kills more US women than any other gynecologic malignancy. More than 75% of new cases present with advanced stage disease that has disseminated into the peritoneal cavity. Primary intervention is debulking surgery, followed by chemotherapy but more than 75% of the women relapse within 5 years. Tumor selective replication competent oncolytic viruses hold promise as a new class of anticancer drugs; they could potentially induce extensive tumor debulking by the ‘oncolytic phase’ followed by the ‘immunotherapy phase’ whereby residual tumor cells are cleared by the host cellular immune cells. We have been developing novel measles viral based therapeutics for the treatment of ovarian cancer. We recently completed a Phase I clinical trial testing the safety and efficacy of oncolytic Edmonston strain measles virus (MV) after intraperitoneal administration into ovarian cancer patients. The virus was well tolerated up to doses of 10^10 TCID_50 repeated for 6 cycles. There was evidence of preliminary biological activity with stabilization of tumor marker CA125 at the higher doses of virus. Strong antitumor T cell responses were observed in some patients. Since virotherapy could benefit from repeat dosing of the virus, there is a need to develop methods for delivery of subsequent doses of virus due to induction of neutralizing antiviral antibodies. To enhance virus delivery and localization in the peritoneal cavity, we have shown in preclinical models the value of using adipose tissue derived mesenchymal stem cells (MSC) as carriers for MV. Toxicology and pharmacology studies in rodents showed that high doses of the cells were well tolerated and did not contribute to tumor growth. Based on these preclinical data, we have obtained approval from the USA Food and Drug Administration to use MSC as carriers of oncolytic MV-NIS in patients with recurrent ovarian cancer. To date, a total of 5 patients have received 10^9 or 10^9 autologous MSC, infected with MV-NIS, safely with no dose limiting toxicities. The dose escalation study is ongoing.

55 GENERATION OF QUANTITATIVE PROTEOMIC AND GLYCOPROTEOMIC PROFILES SPECIFIC TO TRANSFORMED HUMAN ADIPOSE TISSUE MESENCHYMAL STEM CELLS


Multipotent mesenchymal stromal/stem cells (MSCs) are a key component of the tumour environment and play a role in enabling tumour growth. Genetically transformed MSCs may represent the initial cell of sarcoma development. There are currently no reliable methods for discriminating between normal and tumour-promoting human MSCs (hMSCs). Here we aimed at identifying cell-surface membrane glycoprotein markers that are specific to normal and tumourigenic hMSCs and could serve to distinguish each class. We used immortalized/transformed hMSC cultures (n = 3) shown to initiate mixed liposarcoma (MLS) in vivo as a model of human sarcomagenesis and directly compared their membrane protein profile to normal hMSC cultures (n = 3) using quantitative multiplexing proteomics and glycoproteomics. LC-MS analysis was conducted on an Orbitrap Fusion using SPS-MS3.

Quantitative proteomic analysis of normal-cell/malignant cell fractions identified 2700 unique proteins common to all 6 hMSC cultures. Differences in protein abundance levels between each of the 3 normal hMSC cultures (20–100 proteins with levels greater than two-fold change) were found. However, there were profound differences in protein abundance levels between normal and tumourigenic cell classes. The tumourigenic hMSCs exhibited 454 differentially regulated proteins, where 252 proteins displayed a two-fold increase in quantity and 202 proteins displayed a two-fold decrease in quantity. For quantitative glycoproteomics, ~450 unique glycopolypeptides were identified. Preliminary examination showed specific glycoprotein glycoform alterations between hMSC classes. Cellular pathways analysis of (glyco)proteins showing significant quantitative differences between normal and tumourigenic hMSC classes is expected to yield cell-surface membrane proteins that may have utility as biomarkers, as well as non-nuclear membrane and luminal organelle proteins that may shed further light on the biological mechanisms altered in the MLS-initiating cells.

56 INTRAPERITONEAL TRANSPLANTATION OF AMNIOTIC FLUID STEM CELLS SHOW THE THERAPEUTIC POTENTIAL IN EXPERIMENTAL COLITIS

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The colitis or inflammatory bowel diseases (IBD) is increasingly prevalent in recent years due to diet changing and manufactured food up-taking. The stem cell may offer an alternative means to cure IBD or colitis to solve the problems from failed optimal outcome of traditional IBD treatment. With the property of giving rise to three germ layers, expressing pluripotent markers, anti-inflammatory and repairing traits, amniotic fluid derived stem cells (AFSCs) are believed to hold the promise in regenerative medicine. Our group had demonstrated AFSCs could be applied onto several diseases including myocardia infarction and liver fibrosis. In this study, we used pig amniotic fluid derived stem cells (pAFSCs) to treat the experimental model of colitis in mice. Acute colitis was induced in 8 week-old wild type mice by administering 2% dextran sulfate sodium. Then we transplanted 3 million pig Ds-red-harboring amniotic fluid stem cells to the mice with colitis via intraperitoneally injection. The results demonstrated the stem cells transplanted groups having longer colon length compared to untreated groups (colitis mice). In histology study, untreated groups showed significant more inflammatory cells in the lamina propria with cryps damages. The cytokines TNF-α and IFNγ were both found significantly decreased in the stem cell transplanted group. We concluded pAFSC may have benefit for gastrointestinal disorders. The pAFSC showed the ability of inhibition the shortening of colon after induction of colitis, decreasing the inflammatory area with epithelial mesenchymal transformation. Amniotic fluid stem cells could ameliorate experimental colitis in mice and these results might be an potential treatment for the IBD or colitis in the future.

57 SOLUBLE TRAIL-ARMED HUMAN AD-MSC AS NOVEL CELL THERAPY

APPROACH FOR Pancreatic ductal adenocarcinoma


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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive adult tumors and its prognosis is still poor since the number of deaths almost equal the number of new cases. New therapeutic approaches are therefore urgently needed. In our model human adipose mesenchymal stromal/stem cells (AD-MSC) have been armed with a novel soluble TRAIL variant that is constantly released by modified progenitors (sTRAIL-AD-MSC). The wild type TRAIL form is known to act as a tumor inhibitor by a suicide binding site. In this study, by gene engineering, we allow AD-MSC to secrete a trimeric zinc-independent soluble TRAIL variant. The molecule has been then analyzed in vitro and in vivo, either using sTRAIL-AD-MSC supernatant or injecting sTRAIL-AD-MSC cells in a PDAC xenotransplant model. We demonstrated that sTRAIL was stable at 37°C, for at least 24 hours and was able to induce apoptosis in the PDAC lines BxPc-3 and Mia PaCa-2 and, more interestingly, against primary PDAC cells. Moreover, sTRAIL released by AD-MSC...
was able to significantly counteract tumor growth with a reduction of the cytokeratin-7 positive cells and by an anti-angiogenic effect. In parallel, a retrospective study on PDAC specimens form patients (n = 19) has been conducted in order to investigate TRAIL DR4, DR5 and OPG receptor expression in "real" PDAC tissue and generate insights on the possible clinical translation of our approach. Our results suggest that MSC can be vehicles for novel TRAIL variants opening novel opportunities for PDAC treatment by multiple mechanisms.

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58 AUTOMATIC MSC INFUSION IMPROVES EJECTION FRACTION AND WALL THICKNESS IN SEVERE ISCHEMIC CARDIOMYOPATHY. RESULTS FROM A CLINICAL MULTICENTRE PHASE II/III RANDOMIZED CONTROLLED TRIAL

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Background: We have previously demonstrated that mesenchymal stromal cell (MSC) may improve cardiac function and reduce scar tissue in end-stage cardiomyopathy when administered concurrently with revascularization (either cardiac bypass operation or coronary angioplasty). In this study we compared the effects of MSC with concurrent revascularization (Group A), MSC only (Group B) and revascularization only (Group C).

Methodology: Twenty-seven patients were recruited. All patients had anterior myocardial infarction previously and baseline cardiac function (left ventricular ejection fraction, LVEF) less than 40%. Patients who were suitable for revascularization were divided into Group A or C. Patients who have had revascularization previously or were unsuitable for revascularization were allocated to receive MSC by intracoronary infusion (Group B). Patients received between 50-100 x 10^6 autologous bone-marrow MSC. The LVEF, LV and diastolic diameter (LVEDD) and interventricular septum thickness (IVST) were estimated at baseline, 3 months, 6 months and 12 months follow-up. Magnitude of change in LVEF (AEF) was calculated as percentage of baseline value.

Results: All patients tolerated the procedure well with no proarrhythmia, complication or tumor formation. There was no difference in baseline parameters between Groups A, B and C including LVEF (27.5 ± 5.6 vs. 32.0 ± 4.5 vs. 28.0 ± 8.3%; P = 0.26). LVEF improved in all groups during follow-up. The improvements were statistically significant compared to baseline for Group B at 3 months and for Groups A and C at 6 months. The AEF was largest in Group A compared to Groups B and C at 12 months (18 ± 83 vs. 46 ± 17 vs. 31 ± 29%; ANOVA P = 0.02). IVST improved in Group B while LVEDD improved in Groups B and C.

Conclusion: MSC restores myocardial myocardial wall thickness and cardiac function. Concurrent MSC administration with revascularization appeared to be superior to either procedure alone for patients with ischemic cardiomyopathy.

59 SYSTEMIC DELIVERY OF siRNA-BASED THERAPEUTICS USING FUNCTIONALISED SINGLE-WALLED CARBON NANOTUBES

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Carbon nanotubes (CNTs) are potential candidates for drug, antigen and nucleic acid delivery vehicle in nanomedicine. The large surface of CNTs provides structural advantages and allows loading of functional groups or therapeutics such as nucleic acid, drugs and proteins. Our study is to deliver siRNA to cells using single walled carbon nanotube (SWNT) to achieve gene silencing effect. SWNT was functionalized by dissolving one mg of HPC-P@SWNTs and 5 mg of PL-PEG-NH2 or PL-PEG-maleamide in 5 ml of water, sonicated for 60 min at room temperature and centrifuged for 6 h. The supernatant were collected and measured their concentration at 808 nm by UV-VIS-NIR spectrometry. The resulted non-covalent functionalized SWNTs were further conjugated with a 5'-thiolated siRNA against GFP (sgf) and RFP (sirFP). In in vitro silencing of GFP and RFP expression by SWNT-siRNA were evaluated in stable expression cell lines by fluorescence spectroscopy. A range of 50-80% GFP expression knocked down was observed in H1299, HeLa, MCF-7 and 293T cells by SWNT-siGFP. SWNT conjugated with both siGFP and sirFP were shown knocking down both GFP and RFP simultaneously in H1299 stable co-expression cell line. Also, gene silencing was observed despite incubation with inhibitors on different cellular internalization pathways. They are chlora promazine for clathrin-mediated endocytosis inhibitor, genistein for caveolae-mediated endocytosis inhibitor and sodium azide for energy depletion agent. The successful knockdown of GFP expression in different cell lines indicated that siRNA were released from the conjugated SWNT-siRNA in the cytoplasm and silent the gene expression. It is also indicated that two different types of siRNA targets could be conjugated with SWNT and achieved two different gene silencing effects simultaneously. We also found that the internalization of SWNT by the non-phagocytes cells (H1299) did not solely depend on single cellular entry pathway to achieve the gene silencing effect.

60 INTERLEUKIN-6 SILENCING IN MESCOYMYAL STROMAL CELLS BY ADENOVIRUS-BASED SHORT HAIRPIN RNA INHIBITS MULTIPLE MYELOMA CELL GROWTH

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Background: Mesenchymal stromal cells (MSC) produce high levels of interleukin-6 (IL-6) that promotes the growth of multiple myeloma. As current IL-6 monosonal antibody therapies have yet to yield significant clinical responses, more effective method of targeting aberrant IL-6 produc-

Results: In this study, we evaluated the short hairpin RNA (shRNA)-mediated silencing of IL-6 in MSC and the efficacy of these modified MSC on U266 multiple myeloma cell growth inhibition in vitro and in vivo.

Methods: IL-6 shRNA adenovirus vector (pAD-BLOCK-IT/IL6), at Multiplicity of Infection of 20, was transduced into 2x10^6 MSC. Supernatant post transduction was collected at fixed intervals and IL-6 level was determined using ELISA. Viability, immunophenotypic profile and trilineage differentiation capacity of transduced MSC were then assessed. For in vitro efficacy assay, conditioned medium from transduced MSC were added into cells containing 3 x 10^6 U266 at 2:1 ratio. Viability post co-culture was determined at fixed intervals using MTS assay. The in vivo efficacy assay was then evaluated in a murine subcutaneous model of human multiple myeloma followed by histological analysis of the harvested tumours.

Results: At 120 h post transduction, IL-6 was suppressed to 39% at MOI = 20 when compared to control MSC (100%) without affecting MSC major biological properties. In vitro results showed significant inhibition of U266 cell growth by half at day 5 when cultured in conditioned medium of transduced MSC while in vivo results showed significant reduction of U266 tumour volume and tumour mitotic index when co-injected with transduced MSC.

Conclusion: MSC post shRNA-mediated IL-6 silencing displayed in vitro and in vivo antitumour efficacy against multiple myeloma cells. The potential of MSC for stable gene suppression using adenovirus-based shRNA transduction should be further investigated as an alternative approach for targeting IL-6 in multiple myeloma therapy.