

## CELL-BASED TREATMENT MODALITY OF URINARY INCONTINENCE WITH HUMAN MESENCHYMAL STEM CELLS IN A NUDE RAT MODEL

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**Introduction & Objectives:** Cell-based treatment of urinary incontinence by injection of myoblasts into the rhabdosphincter is a recent treatment modality to repair damaged sphincteric myofibers or to restore myofiber formation. Bone marrow-derived mesenchymal stem cells (MSCs) have the capacity to differentiate into muscle cells. The aim of the study was to examine myogenic differentiation of human bone marrow-derived MSCs and to monitor their integration into the urethral sphincter in a nude rat model.

**Material & Methods:** Human MSCs were isolated by plastic adherence from bone marrow and grown to confluence. To induce myogenic differentiation, MSCs in culture passage (P) 1 were exposed to 5-azacytidine (AZA) for 24 hours. Expression of smooth muscle and skeletal muscle antigens was examined both in differentiation-induced MSCs in P2 to P6 and in MSCs not exposed to AZA in P0 and P1 by immunocytochemistry. Monoclonal antibodies against smooth muscle alpha-actin (clone 1A4, clone CGA7), skeletal muscle MyoD (clone MoAb5.8A), and skeletal slow muscle myosin (clone NOQ7.5.4D) were used. For in vivo tracking, detached MSCs were labelled with PKH26, a red fluorescent cell linker. PKH26-labelled human MSCs were directly injected into the bladder neck of athymic nude rats. Integration into the host tissue was monitored histologically after 4, 8, 12, 28, and 56 days of cell injection.

**Results:** Muscle antigens were expressed homogeneously in MSCs after exposure to AZA in all five culture passages investigated. The anti-smooth muscle antibodies 1A4 and CGA7 and the anti-skeletal muscle MyoD antibody MoAb5.8A identified 86-97%, 81-100% and 63-100%, respectively of the MSCs. Undifferentiated MSCs in P0 stained positive in 62%, 60%, and 73%, respectively compared to 94%, 100%, and 92%, respectively for MSCs in P1. There was no reactivity with the antibody NOQ7.5.4D in both groups of MSCs. Histology demonstrated well-defined clusters of fluorescent cells in bladder neck tissues from four until 56 days after cell injection.

**Conclusions:** The results stress the potential of bone marrow-derived MSCs to differentiate into smooth and skeletal muscle cells. An exposure to AZA is not necessary. Survival and integration of MSCs in bladder tissue for up to eight weeks may lead to final differentiation in vivo and fully-functional muscle cells. This model emphasizes the use of autologous MSCs for a cell-based treatment modality of urinary incontinence.

## NOVEL LONGITUDINAL STUDIES OF RAT BLADDER REGENERATION

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**Introduction & Objectives:** Interest in bladder tissue engineering for clinical applications has risen dramatically over the past 15 years. Most commonly grafts made of animal-derived or synthetic materials have been used for bladder augmentation, and recently the first antillogous tissue-engineered neo-bladder has been successfully implanted in patients. Despite these important advances, there is still little known about de novo bladder regeneration in vivo. As a first step in this direction, we have begun to more completely characterize the normal regenerative process of the bladder in vivo. The ultimate goal is to leverage these findings in order to further increase/enhance the urologic applications of tissues engineering/regenerative medicine.

**Material & Methods:** Female F344 rats underwent subtotal cystectomy (STC; removal of >70% of the bladder), and the regenerative process that followed was monitored in several ways. In vivo urodynamic studies were performed on animals up to 15 wks post-ST. High-resolution Micro CT scans were also performed at 0, 1, 2, 4, and 8 weeks post-STC, and linked to urodynamic studies on the same animal. After euthanasia, the contractility of the bladder wall was further characterized with in vitro pharmacologic/physiologic studies. Immunohistochemistry was also performed in order to identify specific cell types involved in the regenerative process.

**Results:** Cystometric analysis showed that bladder volume at time zero was 0.85 ± .09 ml which decreased to 0.39 ± 0.04 ml at 2 weeks post-STC, then gradually increased to 0.47 ± 0.07 ml and 0.71 ± 0.08 ml at 6 and 15 weeks, respectively. Bladder volume calculated from Micro CT scans confirmed the cystometric findings for the same rat. Micturition pressure was significantly higher in the age-matched controls (AMC) at all time points (79 ± 9 vs 45 ± 8 cm H<sub>2</sub>O). Contractile responses of isolated tissue strips derived from regenerating bladders also decreased following STC. Immunohistochemistry staining with c-kit (CD117) showed positive results in the detrusor denoting a possible role for stem cells in regeneration of the muscle layer.

**Conclusions:** Through continued investigation, the combination of high resolution imaging modalities with direct measures of bladder function in vivo and tissue function and histology in vitro will establish the baseline characteristics of bladder regeneration as well as noninvasive markers for physiological milestones associated with normal bladder regeneration. The power of this approach stems from comparison of the regenerated bladder with the native bladder from the same animal. The over-riding goal is to leverage the novel insights obtained to maximize the body's potential for achieving enhanced/maximal tissue formation in vivo.

## THE EFFECT OF HUMAN MUSCLE DERIVED STEM CELLS (MDSC) AND GLYCINE-ISOLEUCINE-LYSINE-VALINE-ALANINE-VALINE (GIKVAV) ON THE CRYO-INJURED BLADDER OF NUDE MOUSE

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**Introduction & Objectives:** In neurogenic bladder, both smooth muscle contraction and nerve regeneration are very important for the functional improvement. GIKVAV is a peptide which can induce nerve regeneration in vivo. In this study, we evaluated the bladder function after MDSC and GIKVAV injection into the cryo-injured bladder of nude mouse.

**Material & Methods:** Human muscle samples were obtained from the rectus abdominis muscle of 12 patients underwent laparotomy. The purpose and entire method of the study were explained to the patients. All the subjects who participated in this study provided written informed consent. The MDSCs were isolated by a modified preplate technique, and only CD34+ human muscle stem cell were extracted by Mini-MACS kits. The nude mice were subdivided into 5 groups (n= each group): normal group (N), saline injection group after cryo-injury (S), GIKVAV injection group after cryo-injury (G), human MDSC injection group after cryo-injury (M) and GIKVAV and human MDSC injection group after cryo-injury (GM). At 2 weeks after injection, we compared the contractility of bladder muscle strip of each group by organ bath and polygraph using electronic field stimulation (EFS). Nerve regeneration was evaluated by choline acetyl transferase (ChAT) immunostaining.

**Results:** The contractile powers of N, S, G, M and GM groups were 3.58±0.27, 1.54±0.25, 1.54±0.31, 2.49±0.36, 2.44±0.34 mN/mg by EFS. The contractility of bladder muscle strip in S and G groups were decreased than N group. The contractile powers of M and GM groups were decreased than N group, but greater than S and G groups. In immunohistochemical staining of ChAT, nerve regeneration was increased in G & GM groups compared with S and M groups.

**Conclusions:** Nerve regeneration was induced by GIKVAV injection regardless of human MDSC injection. There was no direct effect of GIKVAV on bladder muscle contractility.

## UPREGULATION OF L-TYPE CA<sup>2+</sup> CHANNEL ACTIVITY BY THE VDR AGONIST ELOCALCITOL IN HUMAN AND RAT BLADDER

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**Introduction & Objectives:** Uncoordinated bladder myogenic activity leads to symptoms, often complained by aging individuals, known as overactive bladder (OAB) syndrome. Human bladder contraction mainly depends on activation of Ca<sup>2+</sup> influx, via L-type voltage-gated Ca<sup>2+</sup> channels, and on RhoA/Rho-kinase signalling, a calcium sensitizing pathway up-regulated in OAB. Elocalcitol (Elo) is a Vitamin D receptor (VDR) agonist inhibiting RhoA/Rho kinase signalling in rat and human bladder, shown to ameliorate OAB symptoms compared to placebo in a phase II clinical study. Since in normal bladder from Sprague-Dawley (SD) rats Elo treatment delayed the contractile response to carbachol without changing maximal effect, we hypothesized, based on increased sensitivity to the selective L-type Ca<sup>2+</sup> channel antagonist isradipine in bladder strips from Elo-treated rats, an up-regulation of L-type Ca<sup>2+</sup> channels. Thus, we investigated the effects of Elo on bladder smooth muscle L-type Ca<sup>2+</sup> channels.

**Results:** In human bladder smooth muscle cells (hBCs), Elo (10<sup>-12</sup>-10<sup>-7</sup>M) induced a dose-dependent rapid increase in intracellular [Ca<sup>2+</sup>]<sub>i</sub>, with maximal effect at 10<sup>-7</sup>M. This increase was abrogated by pre-treatment with the specific L-type Ca<sup>2+</sup> channel antagonist verapamil, unaffected by thapsigargin, and undetectable in the absence of extracellular Ca<sup>2+</sup>, suggesting an influx through L-type Ca<sup>2+</sup> channels. In whole-cell patch-clamp electrophysiological studies, hBCs exhibited voltage-activated Ca<sup>2+</sup> currents (I<sub>Ca</sub>), T-type and L-type (I<sub>Ca,L</sub>). Accordingly, both isradipine and verapamil only blocked the slow I<sub>Ca,L</sub>, which was enhanced by the selective L-type Ca<sup>2+</sup> channel agonist Bay K8644 (Bay). Addition of Elo (10<sup>-7</sup>M) increased I<sub>Ca,L</sub> size and specific conductance (Gm/Cm), by inducing faster activation and inactivation kinetics than control and Bay, and determined a significant negative shift of the activation (V<sub>a</sub>) and inactivation curves (V<sub>h</sub>), as Bay. In long-term treated hBCs Elo (10<sup>-8</sup>M, 48h) caused a further significant shift of V<sub>a</sub> and V<sub>h</sub> towards more negative potentials and a significant increase of both I<sub>Ca</sub> amplitude and Gm/Cm, which were abrogated by verapamil. Long-term treatment in hBCs also showed that Elo (10<sup>-8</sup>M, 48h) induced mRNA and protein expression, detected by real time RT-PCR and western blot analysis, respectively, of pore forming L-Type <sup>1</sup>C subunit. In bladder strips from SD rats, Bay induced a dose-dependent increase in tension. Interestingly, the maximal contractile effect of Bay was significantly enhanced in bladder strips from Elo-treated rats (30 µg/kg/day, 2 w), with Emax increasing from 40.6±1.9 to 117±6.4.

**Conclusions:** In conclusion, Elo is able to upregulate Ca<sup>2+</sup> entry through L-type Ca<sup>2+</sup> channels in human bladder smooth muscle cells, thus balancing its inhibitory effect on RhoA/Rho kinase signalling, and providing a mechanistic basis for the possible use of this drug in the treatment of OAB.