FINAL ID: S1

TITLE: Inflammatory Enhancement of Colonic Organoids to Study the Anti-inflammatory Effect of Mesenchymal Stem Cells

ABSTRACT BODY:

Purpose/Background: Mesenchymal stem cells (MSCs) have shown clinical efficacy in patients with Crohn's disease. However, little is understood about the underlying mechanism of action. This study aimed to investigate the underlying mechanism of MSCs' effect on preventing and treating inflamed colonic tissue using a patient-derived organoid model.

Methods/Interventions: 3D intestinal organoids were cultured from healthy donor colonic tissue up to passage 4, To mimic an inflammatory microenvironment, 20ng/ml of cytokines (TNF-α, IFN-γ, and IL-1β) were added to the organoid culture media for 24 hours; one organoid culture was organoid alone, the second had cGMP manufactured bone marrow-derived MSCs ($2*10^5/cm^2$) already present (injury prevention), and the third culture had MSCs added after 24 hours for an additional 72 hours at a concentration of ($2*10^5/cm^2$) for the treatment of inflammation. NGS analysis and proteomics assay were performed for studying the post-translational modification effect of MSCs. Immunohistochemistry and immunofluorescent staining were performed for epithelial and inflammatory markers. The lysate and media of the co-cultured organoid with MSCs were analyzed for pro-inflammatory, cytokine, and chemokine panels with a V-plex human Elisa kit, and significant differences were confirmed by western blot.

Results/Outcomes: In the cohort of organoid + MSC after inflammation, there was significantly higher viability, proliferation, and epithelial cell markers, and has less inflammation compared to organoids alone and organoids + pre-MSC. In the cohort of organoid + MSC after inflammation, the organoid morphology showed a fetal-like shape indicating tissue repair as compared to budding morphology seen in the organoid alone and organoid _pre-MSC. The epithelial marker (Epcam and E-cad) were significantly higher in the organoid +MSC cohort as well, comparatively. The results of cytokines showed that adding MSCs after organoid injury significantly increased the chemokine involved in protecting the inflammatory response, and significantly higher VEGF showed injury repair activation. Increased Claudin-1and decreased Claudin-2 gene and protein levels confirmed the injury repair on tissue effect of MSCs after injury.

Conclusions/Discussion: This organoid injury model shows that the MSCs therapy is not preventative, but has a promising effects in reducing inflammation and injury in colonic tissue

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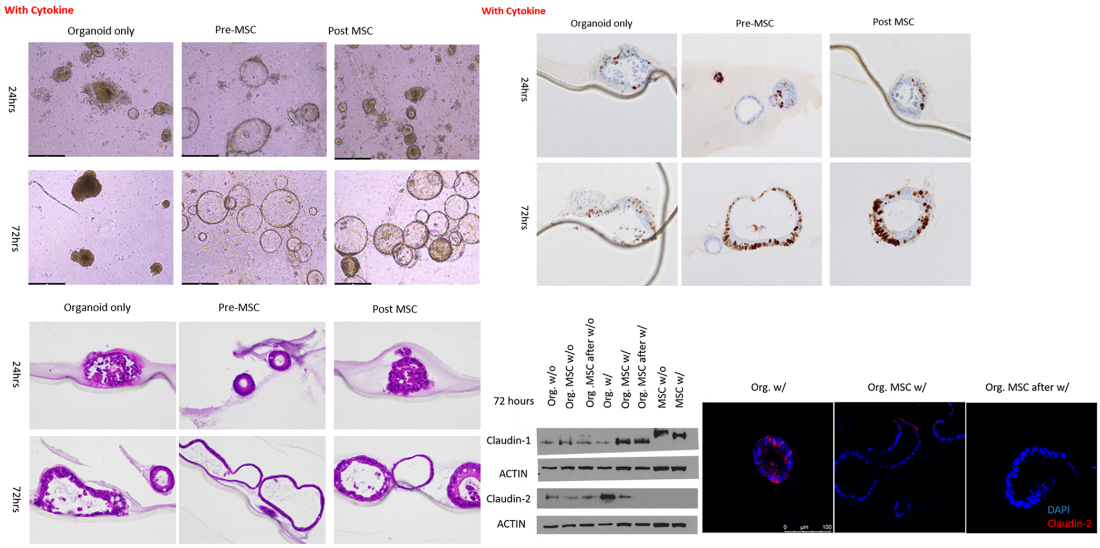

Figure 1- Bright field and H&E images show the morphology changes after MSCs co-culture compared to mono-culture.Ki-67 expression in co-culture is increased compared to organoid only. After hpMSC transplantation, the expression of tight junction protein claudin-1 at the gene and protein levels increased, while the expression of claudin-2 decreased after transplantation, indicating that cell transplantation can promote the recovery of the intestinal barrier.

IMAGE CAPTION: Figure 1- Bright field and H&E images show the morphology changes after MSCs co-culture compared to mono-culture.Ki-67 expression in co-culture is increased compared to organoid only. After hpMSC transplantation, the expression of tight junction protein claudin-1 at the gene and protein levels increased, while the expression of claudin-2 decreased after transplantation, indicating that cell transplantation can promote the recovery of the intestinal barrier.

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FINAL ID: S2

TITLE: Combining Antagonism of IL-23 Along with Immunotherapy Results in Long-term Remission in Preclinical Models of Colon Cancer

ABSTRACT BODY:

Purpose/Background: Patients with colorectal cancer who recieve Immunotherapy suffer from low response rates and high remission despite promising early results. IL-23 in the tumor microenvironment is known to modulate tumor immune response. We sought to find out if combining IL-23 inhibition with PDL1 antagonism would lead to long term remission in murine models of colorectal cancer.

Methods/Interventions: For remission model, a murine colorectal cancer cell line (MC-38) was implanted subcutaneously into C57/B6 mice and divided into following groups (i) isotype control (ii) immunotherapy (anti PD-L1) and (iii) anti-IL-23 antibody in combination of anti PDL1. After treatment for two weeks, only the mice that had complete tumor response were selected and followed for recurrence with periodic tumor measurements. At endpoint, tumors were subjected to single cell flowcytometry.

Results/Outcomes: At the end of two weeks of treatment, a greater proportion of mice that were treated with combination of anti-Il-23 and anti-PDL1 antibody showed tumor regression compared to anti-PDL1 alone (70% vs 50%). Mice with only IL-23 inhibition had no difference in tumor growth compared to isotype control. When only the mice that had complete tumor regression were followed after end of planned treatment of two weeks, mice that recieved combination of anti-PDL1 and anti -IL23 had no tumor recurrence at 15 days of follow up versus 40% recurrence rate in mice who were treated with anti-PDL1 alone. No gross toxicities were identified on combination treatment. Flowcytometry analysis of immune infiltrate to study the change in immune tumor microenvironment showed greater proportion of immune cell infiltration on combination treatment.

Conclusions/Discussion: Combining IL-23 inhibition concurrently with immune check point inhibitor leads to long term remission in preclinical models of colorectal cancer and may hold promise in treatment of patients with advanced colorectal cancer.

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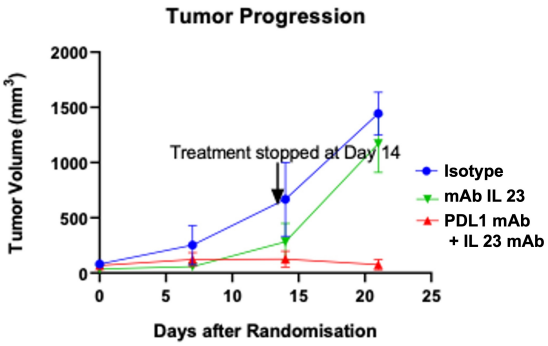

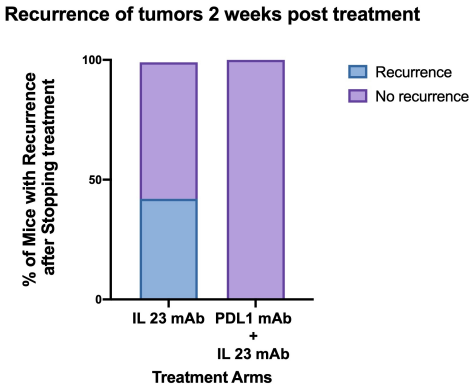

MC-38 colon cancer cells were injected subcutaneously into the backs of WT C57/B6 mice and treated with isotype control (n=5), anti PDL1 antibody (n=8) and a combination of IL-23 and anti-PDL1 antibodies (n-8). Mice that had complete tumor regression after two weeks of treatment were selected and observed for tumor recurrence for two weeks more. Significantly greater proportion of mice treated with anti-PD1 alone had tumor recurrence compared to no recurrence in mice that had received the combination treatment.

IMAGE CAPTION:

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FINAL ID: S3

TITLE: Evaluating Combinatorial Inhibition of Complement and Bcl2 for Early-Onset Colorectal Cancer in Preclinical Models

ABSTRACT BODY:

Purpose/Background: Our group previously demonstrated that the tumor immune microenvironment is distinct between early-onset and late-onset colorectal cancer (CRC) which facilitates tumor progression. Several genes, including complement factor D (CFD), complement component 7 (C7), and Bcl2 were found to have increased expression in early-onset CRC. In gain-of-function experiments, CFD was associated with higher tumor volumes and impacted three genes in mice that were also found to be differentially expressed in early-onset CRC (EGR1, PSMB9, and CXCL9). There is currently a CFD inhibitor (Danicopan) and Bcl2 inhibitor (Venetoclax) approved by the FDA for other indications. We hypothesized that using both a CFD and Bcl2 inhibitor, in combination, would slow growth of tumors and may possibly be used in the treatment paradigm for early-onset CRC.

Methods/Interventions: Ten female athymic nude mice were injected subcutaneously with tumor cells of the HCT-116 colon cancer cell line (derived from an early-onset patient) in the bilateral flanks to allow for growth of two primary tumors per mouse. After allowing for 6-days of tumor growth, the mice were randomized into two groups: five mice were injected daily for 7-days with intraperitoneal combination Danicopan (500μg) and Venetoclax (250μg), while the other five mice were injected with vehicle controls. Tumor volumes were recorded every other day. Experiments were ended on study day 14 and the rates of tumor volume change were compared over time.

Results/Outcomes: Tumor volumes were calculated using length and width measurements obtained by a blinded member of the research team. By study end-point, the mean percent growth in tumor volumes compared to baseline in the control versus experimental groups were 451% and 233% respectively ($p=0.07$), as depicted in Figure 1. The combination of these drugs also resulted in toxicity with a decrease in body weight. Treatment with either drug alone did not result in significant tumor decrease in separate prior experiments.

Conclusions/Discussion: CFD and Bcl2 have previously been shown to be significantly upregulated in early-onset CRC. Our results demonstrate that treatment with a CFD and Bcl2 inhibitor, when used in combination, were able to slow the growth of tumors in a mouse model injected with a human early-onset derived colon cancer cell line. Our study limitations include effects of drug toxicity which might have contributed to early death of two mice in the experimental group prior to study end-point. There was, however, a clear effect with the combination treatment which resulted in slower growth of the tumors. Future studies will include optimizing safe medication dosages and evaluating the combinations in patient-derived and orthotopic models of early-onset colon cancer.

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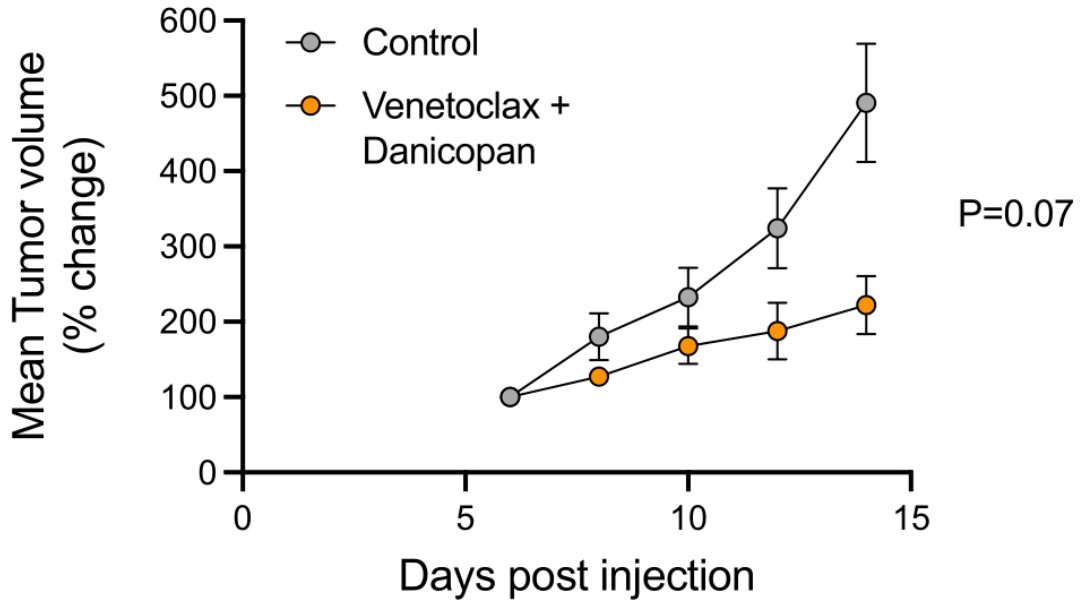

Figure 1. Percent volume change of HCT-116-derived tumors depicted over time after allowing 6-days for tumor growth. By study end-point on day 14, the control group had an increase in tumor volume of 451% and the treatment group had an increase in tumor volume of 233%, p=0.07.

IMAGE CAPTION: Figure 1. Percent volume change of HCT-116-derived tumors depicted over time after allowing 6-days for tumor growth. By study end-point on day 14, the control group had an increase in tumor volume of 451% and the treatment group had an increase in tumor volume of 233%, p=0.07.

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Found 7 Records

FINAL ID: S4

TITLE: Rare Coding Variants in TCHH are Associated with Pilonidal Disease

ABSTRACT BODY:

Purpose/Background: Surgeons have long appreciated the clinical connection between hair and the development of pilonidal disease, but the underlying biology remains unknown. Trichohyalin is a structural protein of the hair follicle and is encoded by the gene TCHH. One known clinical manifestation of deleterious mutations in this gene is Uncombable Hair Syndrome (UHS), a rare disorder of unruly and wiry, although not fragile, hair. It is possible that TCHH plays a role in other hair-related disorders. We hypothesize that rare coding variants in TCHH are associated with pilonidal disease.

Methods/Interventions: We queried whole exomic sequencing data from participants in the Penn Medicine BioBank (PMBB, N = 43,731) for rare coding variants (mean allele frequency <0.1%) within TCHH associated with predicted loss of function (pLOF); specifically stop-gain, stop-loss, frame-shift, or disruption of a canonical splice site. We identified patients with pilonidal disease through association of a diagnostic code for pilonidal disease (ICD9 685, ICD-10 L05) or procedural code for pilonidal surgery (CPT 11770, 11771, 11772). We then examined for an association of pLOF mutations in TCHH with pilonidal disease using Firth's penalized logistic regression controlling for age, sex, and the first ten principal components of ancestry.

Results/Outcomes: In the PMBB, neither pilonidal cysts nor pLOF TCHH varaints were common. Pilonidal cysts were found in 192 (0.4%) patients, and rare TCHH pLOF variants were found in 267 (0.6%) patients. However, among rare variant carriers, pilonidal cyst was more common than among patients without rare variants, 2.6% $p < 0.0003$. Younger age ($p < 0.001$) and female sex ($p = 0.04$) were also associated pilonidal disease. Rare variants included two frameshift variants and three stop-gain variants in TCHH exons 2 and 3. One of the variants has been clinically associated with UHS. Logistic regression revealed that rare variant carriers had an elevated risk of pilonidal disease (OR = 4.81 [95% CI 2.06-11.2]).

Conclusions/Discussion: This small pilot study reveals that carriers of rare, predicted loss-of-function coding variants in the hair structural protein trichohyalin are more likely to develop pilonidal cysts. These early findings help reveal the biological underpinnings behind the long-appreciated connection between hair and the development of pilonidal disease.

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FINAL ID: S5

TITLE: The Impact of Sarcopenia on Surgical Outcomes in Colorectal Cancer - The Interim Analysis of Translational Study Evaluating Clinical Outcomes and Transcriptomic Regulatory Elements of Sarcopenia

ABSTRACT BODY:

Purpose/Background: Sarcopenia, the loss of muscle mass, has been recognised to negatively influence surgical outcomes and reduce oncological survival. However, the regulatory process of sarcopenia is still poorly understood. We sought to study the impact of sarcopenia on surgical outcomes in patients undergoing colorectal cancer curative surgery and to explore the regulatory elements of sarcopenia pathogenesis.

Methods/Interventions: A prospective cohort study was conducted for colorectal cancer patients undergoing elective curative surgery in Sengkang General Hospital since September 2020. Patients were assessed for sarcopenia based on the Asia Workgroup for Sarcopenia 2019 diagnostic criteria.

Details of their surgery and post-operative recovery were collected. Whole blood samples were collected immediately pre-operatively. Rectus abdominis muscles were harvested during their surgery. Satellite cells cell lines were isolated and established. To determine the mechanisms behind sarcopenia pathogenesis, transcriptomic sequencing was performed with the muscle tissue.

Results/Outcomes: Between September 2020 to April 2022, 130 patients were recruited. The incidence of sarcopenia was 35.4% (46/130) with majority (71%, n=33) being male. Sarcopenic patients experiences a marginally longer mean time to gastrointestinal recovery (3.1 vs 2.9 days, p=0.253) and marginally longer mean length of hospitalization group (9.5 vs 9.2 days, p=0.284). The incidence of post-operative complications, defined as Clavien-Dindo ≥3, was slightly higher in the sarcopenic group (6.5% vs 2.4%, p=0.25).

Satellite cells were successfully harvested and cell lines established in all patients. Through transcriptomic analysis of the muscle tissue and cells, pathways involved in immune and development processes such as TNF signalling and inflammatory responses were found to be upregulated in sarcopenic samples.

Conclusions/Discussion: Sarcopenia may result in increased surgical morbidity and lengthens hospitalization. Transcriptomics analysis have highlighted certain pathways implicated in the pathogenesis of sarcopenia. More multi-omics analysis would need to be conducted to fully map the process of sarcopenia to identify targetable regions.

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FINAL ID: S6

TITLE: Development of a Novel Syngeneic Model of Colorectal Liver Metastases

ABSTRACT BODY:

Purpose/Background: With the success of immune checkpoint inhibition in the management of solid organ tumors, immunocompetent pre-clinical models that recapitulate the characteristics of spontaneous colorectal liver metastases (CRLM) are urgently needed to explore therapeutic combinations that invigorate the immune response and maximize the potential benefit of immunotherapy. In the present study, we develop a distinct murine cell line using in vivo selection of CMT-93 cells that reliably and reproducibly results in widespread liver metastases in a timely manner in immunocompetent C57BL/6 mice.

Methods/Interventions: CMT-93 cells were purchased from ATCC (passage <3). Splenic injections were performed using CMT-93 cells in varying concentrations ($1x10^3$ to $1x10^6$ cells) in C57BL/6 mice (6-10 weeks) and allowed to expand for 4 weeks before sacrifice. Metastatic lesions were harvested from the liver and single-cell suspensions were generated. Purification of epithelial tumor cells ($1x10^7$ total cells) was performed using MACS separation kit (Miltenyi Biotec) to exclude heterogenous lymphocyte, fibroblast, and endothelial cell populations from the purified sample. Re-challenge of the established metastatic tumor cell line (metCMT-93) was performed by splenic injection into C57BL/6 mice ($1x10^6$ cells, N=3 mice) and compared to parent CMT-93 cells (N=3) to determine differential uptake. Liver weights and presence of gross metastases were assessed at sacrifice. Transcriptomic variations between parent and daughter cell lines were further explored using qPCR.

Results/Outcomes: Injection of CMT-93 cells into the liver (n=12) of C57BL/6 mice produced only a single, solitary liver metastasis in one mouse after 4 weeks, consistent with prior reports which show engraftment of these cells in C57BL/6 mice is highly inefficient. Isolation of this solitary tumor was performed and free of non-epithelial cell contamination. Re-challenge using splenic injection of metCMT-93 cells induced widespread, bilobar liver metastases in all mice at 3 weeks, whereas CMT-93 cell re-challenge induced no detectable tumor (Fig. 1A). Liver weight was significantly increased in C57BL/6 mice injected with metCMT-93 cells compared to parent CMT-93 cells (1.9 g vs 1.0 g, p=0.045, Fig. 1B). qPCR characterization of this novel cell line demonstrated upregulation in diverse genes involved in epithelial-to-mesenchymal transition.

Conclusions/Discussion: Through in vivo selection, we have developed a syngeneic tumor cell line that can reliably produce CRLM in immunocompetent mice in 3 weeks, enabling the timely evaluation of novel immunotherapeutic approaches in a pre-clinical model. Further downstream characterization of the genomic, transcriptomic, and immunologic characteristics of this model are ongoing in our laboratory.

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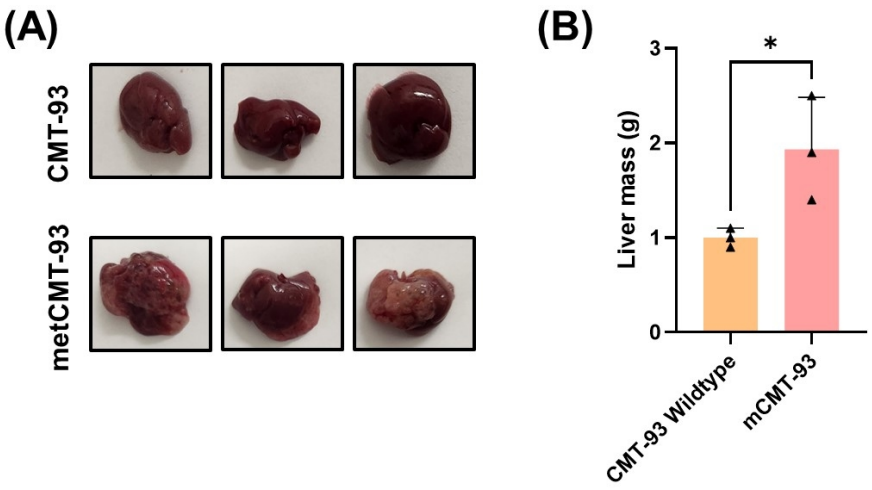
(A)
CMT-93
metCMT-93
(B)
Liver mass (g)
3
2
1
0
*
CMT-93 Wildtype
mCMT-93

IMAGE CAPTION:

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FINAL ID: S7

TITLE: Differentiation of Adipose-derived Stem Cells into Smooth Muscle Cells in an Internal Anal Sphincter-targeting Fecal Incontinence Animal Model

ABSTRACT BODY:

Purpose/Background: Fecal incontinence models targeting smooth muscle cells of the internal anal sphincter have not been reported. Differentiation of implanted human adipose-derived stem cells into corresponding smooth muscle cells in an internal anal sphincter-targeting incontinence model has not been demonstrated. To develop an internal anal sphincter-targeting incontinence model and determine the differentiation of human adipose-derived stem cells into smooth muscle cells in the established model.

Methods/Interventions: Ten Sprague-Dawley rats randomly assigned to cryoinjury and control groups were used to develop an animal model by inducing cryoinjury at the inner muscular layer via posterior intersphincteric dissection. After in vitro confirmation of cell differentiation, 30 rats were randomly assigned to normal, sham cryoinjury, and stem cell-treated groups. Dil-stained stem cells were implanted at the injury site via microscopic needling. Multiple markers were analyzed before implantation and one and two weeks after implantation using hematoxylin and eosin, immunofluorescence, Masson's trichrome staining, and polymerase chain reaction.

Results/Outcomes: We observed stem cell differentiation for smooth muscle cells in the internal anal sphincter-targeting fecal incontinence model. Impaired smooth muscle layers accompanying intact layers were identified in the cryoinjury group. Smooth muscle markers were significantly decreased in the cryoinjury group versus the normal group. In the stem cell-treated group, higher marker levels were observed two weeks after implantation than at one week after implantation. Stem cell tracking revealed that Dil-stained cells were located at the site of augmented smooth muscle bundles, with a diminished population of labelled cells after implantation.

Conclusions/Discussion: We established an internal anal sphincter-specific fecal incontinence model. This study demonstrated that implanted human adipose-derived stem cells restored impaired smooth muscle cells at the injury site, showing stem cell fate corresponding to the established model.

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