Protocol: Transplantation of cells into mouse colon

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CellNet analysis of induced hepatocytes (iHeps) generated from mouse embryonic fibroblasts revealed an intestinal signature in the converted cells. To further explore the intestinal potential of these cells, we utilized a mouse model of colitis and transplanted GFP-labeled iHeps into the acutely damaged colon. iHeps functionally engrafted the colon, integrating into the damaged epithelium and aiding recovery of the animals from their experimentally induced colitis (Morris et al., *Cell* 2014). In this protocol, we describe the method of colitis induction, transplantation of cells, and monitoring of animals. This protocol has been adapted from Yui et al., Nat Med 2012

Part 1. Injury: DSS induced colitis

Supplementing the drinking water of mice with low molecular weight Dextran Sodium Sulfate (DSS) results in colonic epithelial damage accompanied with an inflammatory response. Following 5-6 days of treatment with 2.5% DSS, mice demonstrate the symptoms of acute colitis: bloody stools, diarrhea, and weight loss.

**- ***For cell transplantation studies, we employed immunocompromised *Rag2*$\gamma c^{-/-}$ mice and transplanted cells derived from the Black6 background*

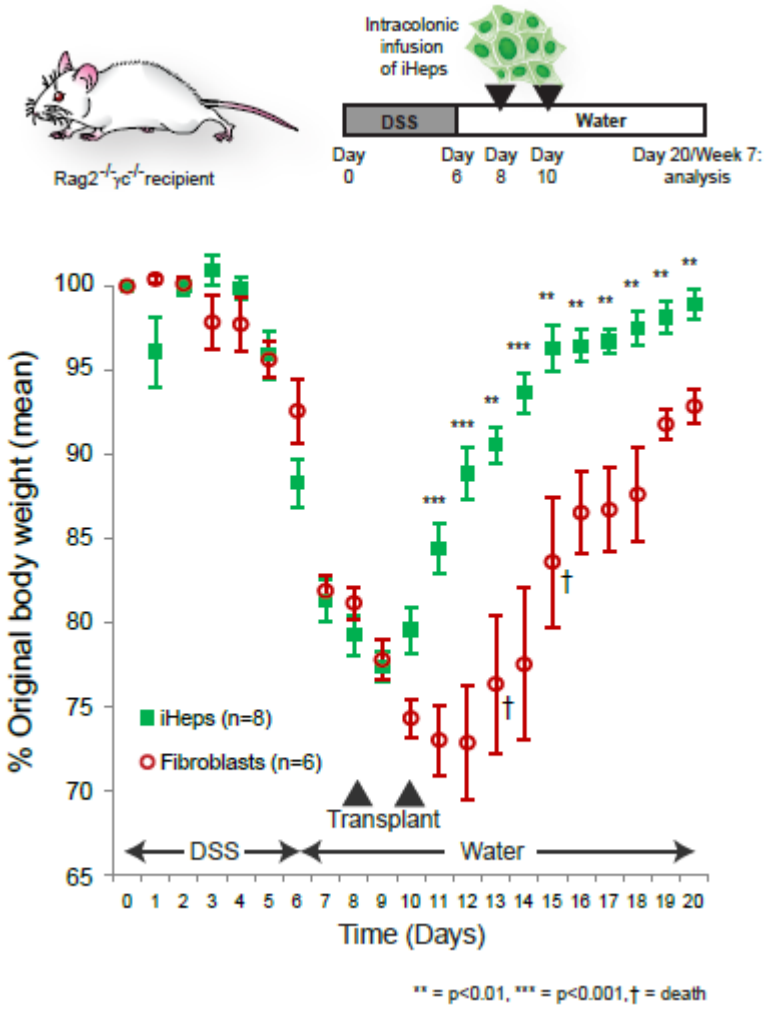
- Mice were monitored for several days prior to DSS treatment to determine their baseline weights.

- A 2.5% (w/v) DSS solution (MP Biochemicals) was made in water and filtered with a 0.45 µm cellulose acetate filter. Note: low molecular weight (MW 36,000-50,000) must be used in order to induce colitis.

- Replace normal drinking water with the DSS water, prohibiting access to regular water.

- After 5-6* days of DSS administration, replace the DSS water with regular water for two days to allow for some repair of the colonic epithelium. *adjust the period of treatment depending on how fast the animals initially lose weight. Weight loss should become apparent 3-4 days following administration of DSS in the drinking water. Refresh the DSS water every 2 days. Replace DSS water with regular water once the animals have lost 5-10% of their original body weight. This protocol was optimized to maximize engraftment of cells into the *Rag2*$\gamma c^{-/-}$ background. Refer to Figure 1 as a guide. Ideally, by the time of the first cell transplantation, the mice should have lost 15-20% of their original body weight.

Figure 1: Outline of the transplantation procedure and typical weight loss/gains following 2.5% DSS treatment for 6 days and transplantation of GFP-labeled fibroblasts (red), or iHeps (green).



Part 2. Transplantation of cells into the colon

- Following DSS treatment, regular drinking water is administered for 48 hours

- At the end of the 48 hour recovery period, prepare the cells for transplantation by harvesting and resuspending in 150ul 1mg/ml matrigel (BD Biosciences) per mouse to be transplanted. We typically transplant 2-5 million iHeps per animal. Keep the cells on ice until transplantation.

- Prepare a 6-8 week old recipient by anaesthetization with isoflurane. Using a 22-gauge plastic angio-catheter (BD Biosciences), infuse the cells into the lumen of the colon slowly and hold the recipient vertically for 3–5 min, maintaining anesthesia while the gel mixture adheres to the colon.

- After 48 hours, repeat the transplantation procedure.

- Monitor weight of the recipient every day for the duration of the experiment. We typically found that recipients reached their original body weight after ~3 weeks after initial DSS administration.

3. Analysis

- We analyze colons for the presence of engrafted cells at 12- and 50-days following transplantation to assess short- and long-term engraftment respectively. Carefully dissect the colon from the euthanized recipient and flush with ice cold PBS, using a 20G feeding needle.

- Prepare the colon for histological analysis by making 'swiss rolls', fixing the tissue in 10% buffered formalin phosphate at room temperature for 24 hrs, replacing with 70% EtOH for an additional 24hrs, followed by paraffin sectioning. A detailed description of this procedure can be found here: http://www.jove.com/video/1652/murine-colitis-modeling-using-dextran-sulfate-sodium-dss

Figure 2: Examples of engrafted colon, 12- and 50-days following transplantation: Immunohistochemistry and immunofluorescence