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MC-02 Increased wound healing in p-glycoprotein deficient intestinal cells

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Abstract

Inflammatory bowel disease (IBD) is an autoimmune disease of unknown cause and includes conditions such as Crohn's disease and ulcerative colitis. With no cure and only palliative therapies available, many patients with these conditions struggle with diarrhea, abdominal pain, and other chronic symptoms. This study is interested in investigating the multidrug resistance gene (MDR) which encodes the small molecule pump P-glycoprotein (P-gp).

This gene is responsible for regulating drug absorption and accumulation in various parts of the body such as the intestines which is of particular interest to this study. Polymorphisms of the MDR1 gene (encoding p-glycoprotein) have consequently been linked to IBD in humans. We hypothesized that MDR1 deficient Caco-2 intestinal cells would heal wounds slower than control Caco-2 cells, resulting in increased leakage between the tight junctions of the intestines and an increase in disease progression.

Surprisingly, The MDR deficient cells have shown increased wound healing compared to control cells. To determine the mechanism by which the MDR deficient cells were healing wounds faster, we investigated cellular proliferation and analyzed the cellular shapes and sizes of the MDR deficient cells relative to control cells. Using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) cell proliferation assay, we detected a difference in proliferation between the MDR deficient and control cells at various time points. We also performed membrane staining which allowed us to visualize and determine the changes in cellular shape and size between the deficient and control cells.

These experimental techniques ultimately revealed changes in proliferation for the MDR deficient cells when compared to the control Caco-2 cells as well as differences in the cellular shapes and sizes of these deficient cells. Currently, we are investigating the integrity of the barrier formed by MDR deficient cells after the wound healing is complete.

The changes we detected suggest that the wound healing mechanism differs when MDR is missing from cells, which, in the intestine, could lead to the development of inflammatory bowel disease.

Methods

Cell culturing: Caco-2 and MDR-deficient (MDR-/-) Caco2 cell lines were obtained from MilliporeSigma. Cells were cultured in DMEM + pyruvate supplemented with 10% fetal bovine serum, penicillin, streptomycin, amphotericin B, and non-essential amino acids. Cells were cultured at 37ºC with 5% CO_2.

Wound healing Protocol: Cells were cultured to confluency and wounded with a 10µl pipette tip. Cultures were allowed to heal for indicated times.

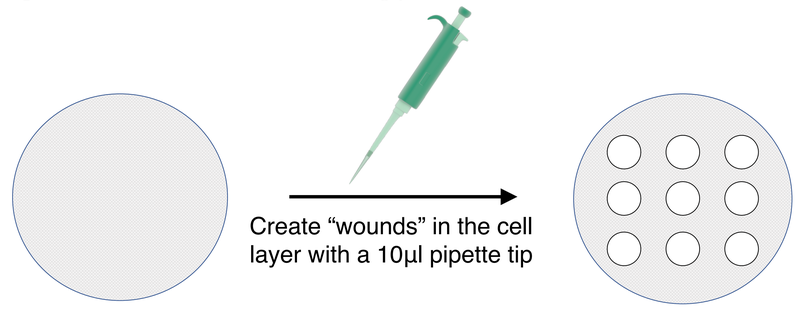
MTT Assay: Cells were cultured and wounded as described above. 24 hours post wound healing, the Biotium MTT Cell Viability Kit. After addition of MTT reagent, cells were cultured for an additional 24 hours. Absorbance was measured at 475nm, with background at 650nm subtracted.

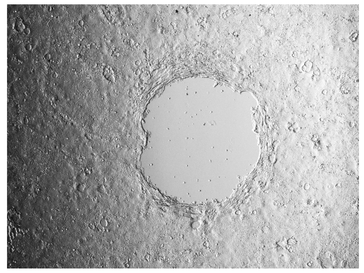
CellTrace Violet Staining: Cells were cultured and wounded as described above. 24 hours post wound healing, CellTrace Violet dye was added to the cells. After labeling for 20 minutes, dye was washed and cells cultured for an additional 24 hours. Cells were released from the culture dish using Trypsin-EDTA, washed, and analyzed using the AttuneNxT flow cytometer using the violet laser.

Statistical Analysis: Statistics were performed using a Student's t Test using GraphPad Prism software

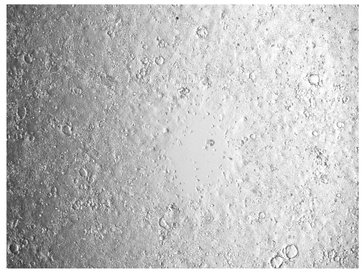
Wounding Protocol

Figure 1: Overview of wound healing protocol.





Wound immediately after wounding (0 hours)
Image by USC Upstate Student Caleb Gosnell



Healing wound (48 hours).
Image by USC Upstate Student Caleb Gosnell

MDR$^{-/-}$ Caco-2 Cells Heal Wounds Faster

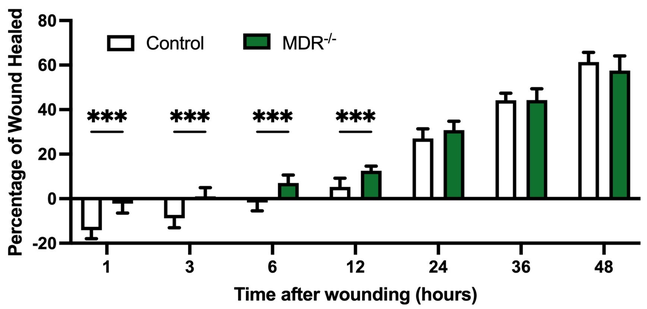


Figure 2: Wound healing rate in MDR$^{-/-}$ Caco-2 Cells is increased. Control and MDR$^{-/-}$ Caco-2 Cells were cultured to confluency and wounded as described above. Phase contrast images were captured at 0, 1, 3, 6, 12, 24, 36, and 72 hours after wounding. Wound area at each timepoint was measured using ImageJ. Percent of wound healed was calculated from the 0 hour area. *** = $p < 0.001$, N = 10 samples/group

Increased metabolic rate in MDR$^{-/-}$ Caco-2 cell Culture after wounding

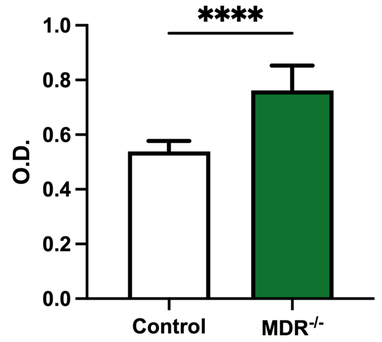


Figure 3: Metabolic rate in MDR$^{-/-}$ Caco-2 Cells is increased. Control and MDR$^{-/-}$ Caco-2 Cells were cultures to confluency and wounded as described above. XTT assay was performed 24 hours after wounding. *** = $p < 0.001$, N = 8 samples/group.

Increased Cell Division in MDR$^{-/-}$ Caco-2 Cells

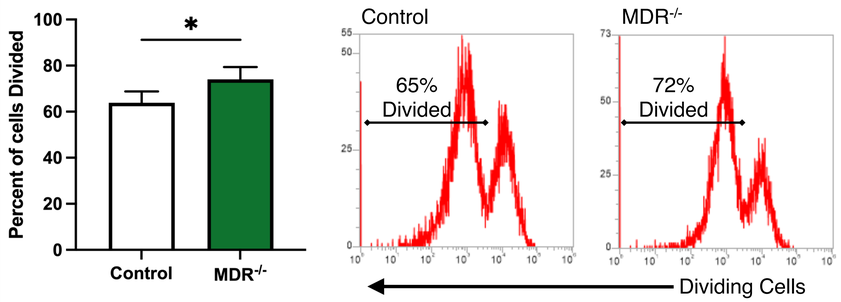


Figure 4: Wound healing rate in MDR$^{-/-}$ Caco-2 Cells is increased. Control and MDR$^{-/-}$ Caco-2 Cells were cultures to confluency and wounded as described above, and labeled with CellTrace Violet. After 24 hours, cells were analyzed via flow cytometry. *** = $p < 0.001$, N = 5 samples/group

Summary/Conclusions

- MDR$^{-/-}$ cells proliferate at a faster rate than control cells
- MDR$^{-/-}$ cells show increased metabolic rate24 hours after healing. This could indicate increased proliferation, leading to more cells present in the culture, or increased metabolism to heal the wound, or a combination of the two mechanisms.
- Increased division using CellTrace Violet/Flow Cytometry shows individual cells division. This suggests the increased wound healing MDR-/- cells is a result of increased cell division.
- Overall, MDR$^{-/-}$ cells have an altered wound healing mechanism that may impact the development of inflammatory bowel disease.

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