Role of Mesenchymal Stem Cells in Intestinal Epithelial Cell Growth

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INTRODUCTION

- Disruption of intestinal homeostasis can lead to inflammatory bowel diseases (IBDs) like ulcerative colitis
- Mucosal healing derived from intestinal epithelial regeneration is crucial in the remission of patients with IBDs.
- Human Bone Marrow Mesenchymal stem cells (HBMMSCs) have been researched as a therapeutic approach to promote mucosal healing through immunomodulation, angiogenesis, and tissue repair. (1)
- Mechanisms that underlie how HBMMSCs function during intestinal epithelial regeneration are still unknown
- Hedgehog (HH) signaling plays a pivotal role in coordinating interactions between epithelial and mesenchymal cells. Specifically, epithelial cells produce HH ligands, which in turn activate target gene transcription in adjacent mesenchymal cells, thereby triggering MSC responses.

STUDY AIM

Understanding how HBMMSCs interact with intestinal epithelial cells to improve epithelial homeostasis and regeneration using a 3D coculture model

HYPOTHESIS

We hypothesized that colonoids cocultured with HBMMSC would increase cell growth and stemness, which are principal for epithelial regeneration.

METHODOLOGY

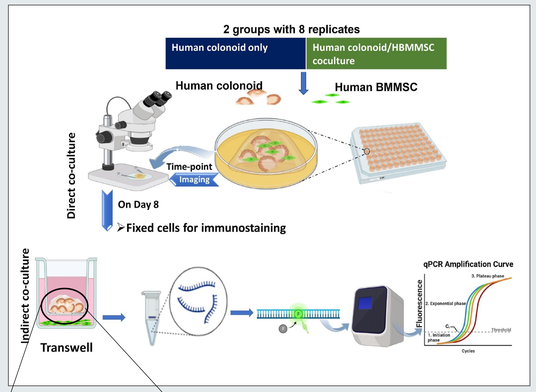
Stage 1

1. First we cultured HBMMSC in flasks until they reached 85% confluence. The cells were grown in specialized HBMMSC media that contained growth factors, antibiotics, and Dulbecco's Modified Eagle Medium as the base.
2. Human enteriods were expanded on collagen plates using conventional intestinal expansion media
3. Both cell types were passaged until they reached desired confluence for subsequent experiments.

Stage 2

1. We designed two experimental models - direct and indirect coculture settings. Direct was used for morphological evaluation. The control group contained colonoid only and coculture included both colonoid and HBMMSC embedded in diluted matrigel.
   - Each group had 8 replicates. The indirect coculture was done using transwell for molecular assays.
   - This design allow us to place the colonoid above the HBMMSC, thereby mimicking the human intestinal architecture.
2. We allowed the cells to grow for 8 days, replenishing the media every two days.
3. On the days that we replaced media, we also collected image samples of the direct coculture and the colonoids only.
4. After 8 days, we fixed the cells for immunostaining to validate the data
5. The indirect coculture was used for qPCR assays to analyze molecular signatures.

EXPERIMENTAL DESIGN



DATA

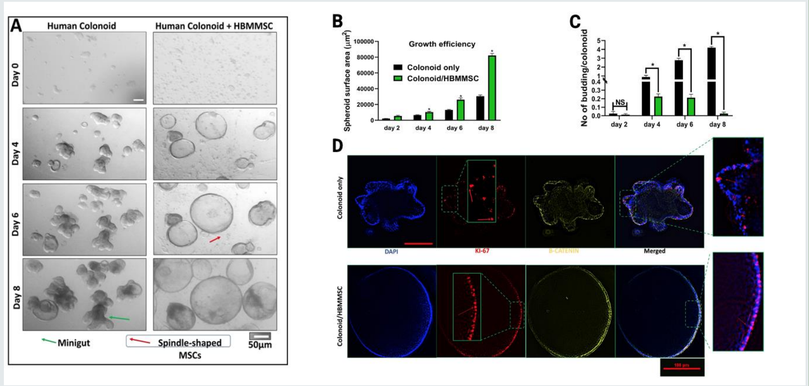


Figure 1: HBMMSC supports human colonoid stemlike morphology. A) Human colonoid representative images. B and C) Quantification of the spheroid surface area and number of budding/colonoids. D) Immunofluorescent staining of antibodies against KI-67 and BETA-CATENIN as markers for proliferation and cell membrane, respectively.

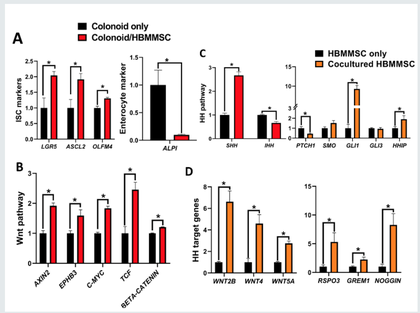


Figure 2: HBMMSC promotes intestinal stemness molecular signatures during normal conditions. Differential gene expression levels of A) Intestinal epithelial cell markers. B) Wnt signaling activities C) Hedgehog signaling in the colonoid and HBMMSC. D) Hedgehog target genes expressed in the HBMMSC. mRNA was isolated from either colonoids only and co-cultured colonoids or HBMMSC of HBMMSC only and cocultured HBMMSC for epithelial or MSC target gene transcription activities.

RESULTS

During normal conditions, the data showed that HBMMSCs in the coculture promotes intestinal stem-like phenotypes compared to the colonoid only groups. The cocultured groups showed enhanced stemness and growth, indicated by increased spheroidal surface area and decreased budding counts. Genes of pro-Wnt signaling ligands (WNT2B, WNT4, WNT5A and RSPO3) were enhanced in the coculture groups. There was also enhanced signaling of stem cell and Wnt target gene expressions (LGR5, AXIN2, ASCL2, C-MYC, TCF, and B-CATENIN). Differentiation markers (ALPi, IHH, and PTCH1) were reduced in the coculture.

DISCUSSION

Using the data we obtained and analyzed, we can infer:

1. HBMMSCs potentiate the stem cell-like phenotypes exhibited by human cocultured colonoids
2. HBMMSCs upregulate stem cell target genes and repress differentiation marker expression
3. HBMMSCs sustain the maintenance of stemness in human colonoids by facilitating the transcription of stemness factors
4. The increased production of intestinal pro-stemness factors by HBMMSCs was triggered by SHH signaling originating from the colonoids

CONCLUSION

The research illustrates the direct interaction between HBMMSCs and intestinal epithelial cells in vitro, mimicking the supportive functions of MSCs in maintaining intestinal stemness under normal circumstances. The developed model offers an enhanced in vitro approach for preconditioning MSCs using HH ligands and other relevant molecules. Crucially, this model is well-suited for future drug screening assays aimed at investigating interactions between intestinal epithelial and mesenchymal cells.

FUTURE RESEARCH

Further research needs to be done to understand the repair mechanisms and cellular pathways that are influenced by the presence of HBMMSC, specifically those that contribute to regeneration. To further investigate the idea that they contribute to healing, a study can be done where colonoids are exposed to injury (heat, hypoxia) and then allowed to heal in both the presence and absence of the HBMMSCs.

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