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Research Advance

Comments on '*MAP3K2-regulated intestinal stromal cells define a distinct stem cell niche*'

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For a long period of time, intestinal mesenchymal stromal cells (IMSC) have been considered as a relatively simple and homogeneous group of cells. These cells could effectively regulate intestinal homeostasis and epithelium integrity via producing growth factors and cytokines (Powell et al., 2011). With the help of single-cell transcriptomics studies, it has now been clear that IMSC are quite complex and heterogeneous (Kinchen et al., 2018). However, the detailed cellular and molecular mechanisms that regulate the function of these cells remain poorly understood. Therefore, the ability to perturb and evaluate the *in vivo* function of IMSC is critical to the understanding of intestinal stem cell niche and the etiology of the inflammatory bowel diseases as well as colitis-associated colorectal cancer.

In our recent work, Wu et al. (2021) found that MAP3K2 protected mice from severe colitis upon dextran sodium sulfate-induced tissue damage via the reactive oxygen species (ROS)–MAP3K2–Krüppel-like factor 2 (KLF2)–R-spondin1 axis (Figure 1). Investigation of *Rspo1*-expressing IMSC revealed that *Rspo1* was only expressed in a fraction of these cells, and MAP3K2 only regulated *Rspo1* expression in a subset of $CD90^+CD81^+CD34^+$ mesenchymal stromal cells but not in $CD34^-$ mesenchymal stromal cells (also *Rspo1*-positive). Further studies on these $CD90^+CD81^+CD34^+$ triple-positive cells with assay for transposase-accessible chromatin using sequencing (ATAC–seq) revealed that they possessed a unique transcriptional regulatory network involving KLF, early B cell factor (EBF), and androgen receptor, as compared to the other three stromal cell populations like $CD34^+$ fibroblasts, $CD146^+$ myofibroblasts, and $CD34^-$ telocytes (Shoshkes-Carmel et al., 2018). Therefore, MAP3K2-regulated intestinal stromal cells (MRISC) are a unique population of IMSC, which protect the colon from acute injury by maintaining the intestinal stem cell niche.

IMSC have already been shown to modulate the proliferation and differentiation of intestinal stem cells. However, due to the lack of precise genetic models, the study in this field has long been dependent on the limited reporter or lineage tracing lines like Col6a1-Cre and Pdgfra-GFP. Thus, the *Rspo1* reporter mice generated in our study (Wu et al., 2021) would help to promote and refine our understanding of these heterogeneous stromal cell populations, especially their function in the immune system, nervous system, tumor biology, and aging. This study also paves new roads towards the stromal regulation of the gastrointestinal, immune, and central nervous systems, both in humans and mice.

Also in this study, we developed a cell delivery technique called orthotopic surgical injection, which allowed us to transfer these $CD81^+$ cells locally into the submucosa. Combined with quantitative polymerase chain reaction and immunofluorescence techniques, we have shown that submucosal delivery of $CD81^+$ cells could indeed regulate the intestinal epithelial cells, indicated by the upregulated expression of the intestinal stem cell marker gene *Lgr5* (Wu et al., 2021).

By using single-cell transcriptomic analysis for IMSC populations, we determined two characteristics of MRISC, besides being valuable resources for researchers who focus on the regulation of intestinal stem cell niche. One is the intestinal mesenchymal lineage precursor-like feature revealed by monocle pseudotime analysis (Wu et al., 2021). Cluster-unique marker gene analysis also revealed that MRISC highly express some previously known mesenchymal lineage precursor marker genes like *Wt1* (Zhou et al., 2008), *Prrx1* (Sanchez-Gurmaches et al., 2015), and *Ebf2* (Wang et al., 2014). There even has been lineage tracing evidence showing that another marker gene of $CD81^+$ stromal cells, *Grem1*, could lead to the labeling of the whole intestinal mesenchymal stroma (Worthley et al., 2015; McCarthy et al., 2020). Furthermore, ATAC–seq and single-cell regulatory network inference and clustering analysis revealed that the mesenchymal stemness-maintaining factor KLF (Jiang et al., 2008) is one of the key master regulators for MRISC (Wu et al.,

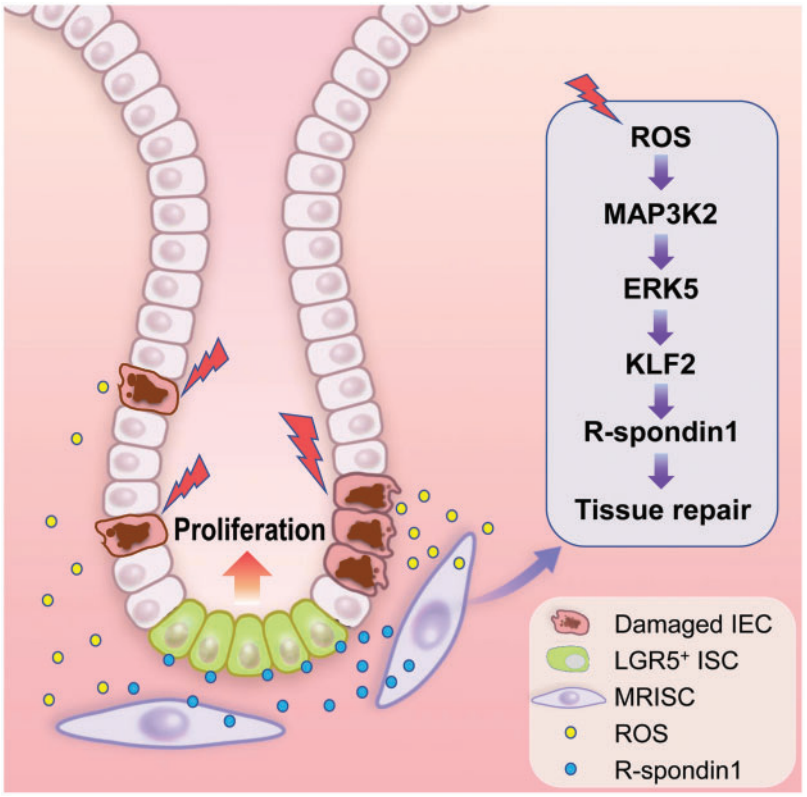

Figure 1 MRISC protect the colon from acute intestinal injury/tissue damage by maintaining LGR5^{+} intestinal stem cells through a ROS–MAP3K2–KLF2–R-spondin1 axis. IEC, intestinal epithelial cell; ISC, intestinal stromal cell.

2021). Meanwhile, although our study and others mainly emphasized the effect of this unique IMSC population in regulating epithelium regeneration, the results also indicated its role in inflammation. Indeed, our scRNA–seq results revealed that MRISC were the only stromal cells that expressed proinflammatory cytokines and chemokines like *Il6*, *Il33*, *Ccl19*, and *Ccl7*. Our immunofluorescence analysis also indicated the unique juxta–lymphatic localization of MRISC, indicating a potential role in regulating immune cell transendothelial migration (Wu et al., 2021). Deeper understanding of complicated functions by this population of cells requires more *in vivo* investigation using cell-type-specific ablation systems like iDTR and more precise genetic tools.

Our unpublished data also indicated that there might be intra-MRISC heterogeneity. For instance, the T cell chemokine, *Ccl19*, was only expressed by a small portion of MRISC, while its molecular sponge was expressed by the rest of this set of cells. Therefore, understanding the intra-MRISC heterogeneity might be key to fully elucidating the true biological nature of MRISC.

Utilization of the tissue-repairing function in regenerative medicine may be a promising application of MRISC. However, it remains difficult to absolutely determine their human counterpart. The most transcriptome-wide similar cells in the human gut are only ~10% similar to MRISC based on the correlation analysis between MRISC and previously reported Str3 cells in humans (Kinchen et al., 2018). Moreover, our study also pointed out the potential downside of this cell population, including its proinflammatory cytokine-producing capability and uncontrollable differentiating potential. Further studies on characterizing the upstream signals that regulate the proinflammatory function and differentiating potential with human counterpart of MRISC would be essential for their clinical applications in treating inflammatory bowel disease patients.

Remaining challenges include: (i) understanding the ontogeny and differentiation of MRISC during development and tissue remodeling; (ii) establishment of MRISC-specific tracking and targeting system to study MRISC plasticity; (iii) characterization of the upstream signal that regulates the proinflammatory function of MRISC; (iv) further interrogation of the heterogeneity within MRISC upon tissue remodeling; (iv) identification of human counterpart of MRISC.

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