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Entitled

INVESTIGATIONS OF THE POTENTIAL USE OF MACROPHAGES IN GASTRIC STEM CELL ACTIVATION, DIFFERENTIATION AND ULCER REGENERATION

by

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Abstract

Stomach erosions and ulcerations are common health problems and their therapy requires tissue regeneration. While current therapy of these disorders focuses on targeting parietal cells to inhibit acid secretion, the role of stem cells in the regeneration process is neglected. In this study, the aim is to investigate the potential use of macrophages to activate gastric stem cells in co-culture experiments and in a mouse model of gastric ulcer. Bone marrow-derived macrophages were prepared and cultured in the presence of lipopolysaccharides and interleukin-4 to respectively induce their differentiation into pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. Polarization of these phenotypes was confirmed by the expressions of IL-1β, IL-6 and iNOS (for M1) and Arg1, Fizz1 and Ym1 (for M2). Mouse gastric progenitor/stem cells or organoids were co-cultured with macrophages or treated with their conditioned media to study gastric epithelial differentiation. In addition, mice with acetic acid-induced gastric ulcers were injected with M1, M2 or M1 plus M2 macrophages to test their possible effects on gastric mucosal regeneration. Combined lectin- and immuno-cytochemistry revealed that M2 conditioned media treatment and co-culture of M2-macrophages with gastric progenitor/stem cells or organoids stimulated the production of mucous neck cells and parietal cells. In addition, RT-qPCR analysis showed that while Muc6, HK-ATPase and CgA expressions were significantly increased in M2 co-culture, differentiation markers were downregulated in M1 co-culture. In acetic acid-induced gastric ulcer tissues, proliferating epithelial cells were observed at the base of regenerating glands. Macrophage injections increased the number of macrophages and reduced the size of ulcer area by day 7. While M1 injections activated Wnt signaling pathway and was associated with enhanced cell proliferation and ulcer healing, no sign of differentiation was observed. On the other hand, profiler PCR arrays showed that M2 injections induced TGF-β/BMP, Wnt, Hedgehog and Notch signaling pathways, and enhanced both proliferation and differentiation of epithelial cells. M1 plus M2 injections also showed increase in epithelial differentiation. This study reveals the beneficial role of macrophages in gastric progenitor/stem cell activation and ulcer regeneration which might lead to future clinical applications.

Keywords: Stem cells, gastric gland, macrophages, gastric ulcer, regeneration