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ESOPHAGEAL ORGANOID PROLIFERATION AND DIFFERENTIATION ARE ALTERED BY LOSS OF MSH2

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Background: The stratified epithelium of the esophagus includes *Krt15+* basal stem cells that display self-renewing and regenerative capacity, and multipotency. However, the mechanisms that specifically control their functions remain unknown. Interestingly, RNA sequencing and GSEA revealed an enrichment of a gene set associated with DNA repair in *Krt15+* cells vs *Krt15-* cells. We also observed that *Msh2* (DNA mismatch repair pathway) is the most significantly upregulated gene in *Krt15+* stem cells.
Aims: To determine the effect of Msh2 loss on self-renewal and differentiation of esophageal organoids.
Methods: Esophageal epithelial cells were isolated from a wild-type mouse. Using flow cytometry, esophageal *Krt15+* (GFP+) and *Krt15-* (GFP-) cells were sorted from *Krt15-CrePR1 ($R26^{mT/mG}$)* mice. All cell populations were grown as organoids and *Msh2* was depleted using a CRISPR/Cas9 approach. Impact of *Msh2* loss on self-renewal and differentiation in esophageal epithelial organoids was evaluated through organoid formation assays, WST-1 proliferation assays and histological analysis.
Results: At baseline, organoids depleted for *Msh2* formed more poorly differentiated and less well-differentiated organoids than controls. Lower expression of differentiation gene *Krt13* was also observed in *Msh2*-depleted organoids, confirming an altered differentiation pattern. Furthermore, these organoids showed a higher organoid formation rate and proliferation by WST-1 assay, suggesting that self-renewal capacity and viability are increased when *Msh2* is depleted. Interestingly, following radiation, organoids depleted for *Msh2* showed higher residual levels of p-H2AX (DNA damage marker), suggesting that their capacity to cope with DNA damages is altered. As mentioned above, we previously reported that *Msh2* is the most upregulated gene in *Krt15+* vs *Krt15-* cells. Therefore, to determine if Msh2 role is distinct in both populations, we depleted *Msh2* in *Krt15+* and *Krt15-* cells-derived organoids. Interestingly, our preliminary results suggest that *Msh2* deletion led to increased p-H2AX and decreased Krt13 levels in *Krt15+* organoids but not in *Krt15-* organoids.
Conclusions: Our results show that Msh2 is potentially a key contributor of esophageal stemness in homeostatic and injured conditions.

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