

 COLORECTAL CANCER

Fibroblasts orchestrate tumour initiation

Colorectal cancers (CRCs) originate from epithelial stem cells in intestinal crypts but whether the surrounding mesenchymal cells contribute to tumorigenesis is not known. Roulis, Kaklamanos et al. conducted single cell RNA sequencing (scRNA-seq) in mice to characterize intestinal mesenchymal cells and in doing so found a population of rare fibroblasts present in intestinal crypts that promote intestinal tumorigenesis in a paracrine manner.

Several populations of fibroblasts were identified through scRNA-seq, and pathway analyses of these cells indicated that two populations had enrichment of arachidonic acid metabolism. This pathway is strongly associated with CRC: inhibition of prostaglandin-endoperoxide synthase 2 (PTGS2, also known as cyclooxygenase 2 (COX2)), an enzyme in the pathway, prevents CRC, although the molecular mechanism for this is not known. Immunostaining in mouse intestines indicated that one of the fibroblast populations with high PTGS2 levels was a population known as Cajal cells in the muscular layer. The second was a rare population located close to the stem cell zone of intestinal crypts; the authors termed these cells rare pericryptal PTGS2-expressing fibroblasts (RPPFs).

Fibroblast-specific knockout of *Ptgs2* in mice indicated that fibroblasts are the predominant source of PTGS2 in intestines. To determine the role of this pathway in tumour formation, *Apc^{Min/+}* mice (which spontaneously develop intestinal tumours) were crossed with mice lacking *Ptgs2* in fibroblasts. These *Apc^{Min/+};Ptgs2^{ΔFibr}* mice had a reduction in tumour formation, but there was no difference in the size of tumours that did form, indicating that PTGS2 in fibroblasts is important for tumour initiation but not growth. Similar results were observed using two additional mouse models: one in which *Ptgs2* expression in *Apc^{Min/+}* mice was

“ fibroblast-derived PGE₂ might be responsible for intestinal tumour initiation ”

induced exclusively in fibroblasts and one in which intestinal tumorigenesis was driven by injections of the mutagen azoxymethane in *Ptgs2^{ΔFibr}* mice.

Lipidomic analyses indicated that there was a reduction of prostaglandin E2 (PGE₂) and prostaglandin I2 (PGI₂) secretion in intestines of *Ptgs2^{ΔFibr}* mice compared with control mice. PGE₂ has a known tumour-promoting role, so the authors hypothesized that fibroblast-derived PGE₂ might be responsible for intestinal tumour initiation in their mouse models.

To look more closely at the role of PGE₂, the authors cultured intestinal crypts from wild-type mice in organoid growth media (OGM) in the presence of exogenous PGE₂. This led to the growth of spheroids rather than typical budding organoids with a crypt-like structure, indicating a lack of differentiation and increased stem cell-like behaviour. They determined that the key receptor for PGE₂ in intestinal epithelial cells was PTGER4, and OGM cultures of crypts lacking *Ptger4* in intestinal epithelial cells (*Ptger4^{ΔIEC}*) remained differentiated in the presence of PGE₂. Similarly, spheroids grew when control

but not *Ptger4^{ΔIEC}* crypts were co-cultured with fibroblasts in OGM. Spheroid formation was also blocked by a PTGER4 inhibitor. scRNA-seq supported the increased stem cell function of epithelial cells in response to signalling through PTGER4; importantly, this pathway induced expansion of a population of cells that typically functions as reserve stem cells (RSCs).

PTGER4 activation induced transcription of YAP1 target genes, and deletion of *Yap1* from intestinal crypts (*Yap1^{ΔIEC}* crypts) prevented spheroid formation in the presence of fibroblasts in OGM. Furthermore, RSC expansion was prevented in *Yap1^{ΔIEC}* crypt and fibroblast co-cultures. Stimulation of wild-type organoid cultures with PGE₂ induced YAP1 dephosphorylation, nuclear translocation and downstream transcription, which were blocked by a PTGER4 inhibitor. YAP1 nuclear localization was also observed in early stage adenomas in *Apc^{Min/+}* mice, as was expansion of a population of cells expressing SCA1 (an RSC marker and YAP1 target gene). This was not observed in *Apc^{Min/+};Yap1^{ΔIEC}* mice or *Apc^{Min/+};Ptger4^{ΔIEC}* mice, and these mice developed fewer tumours. As with the authors' earlier mouse models, the few tumours that did form did not have reduced growth, again indicating that tumour initiation, but not growth, is affected by this pathway.

The authors demonstrated that organoid cultures of crypts isolated from normal human colon tissue formed spheroids in the presence of PGE₂, and this was blocked by a PTGER4 inhibitor. Furthermore, YAP1 immunostaining in tissues from patients with colorectal tumours indicated that YAP1 is nuclear in tumours but not neighbouring normal tissue.

This study has illuminated a fibroblast-driven molecular pathway by which tumour initiation might occur. As COX2 inhibitor toxicity has prevented their use in cancer prevention and PTGER4 inhibitors are available, it will be especially interesting to determine whether PTGER4 inhibitors might be used for preventing CRC development.

Sarah Seton-Rogers

ORIGINAL ARTICLE Roulis, M. et al. Paracrine orchestration of intestinal tumorigenesis by a mesenchymal niche. *Nature* <https://doi.org/10.1038/s41586-020-2166-3> (2020)

Credit: Philip Patenall/Springer Nature Limited

