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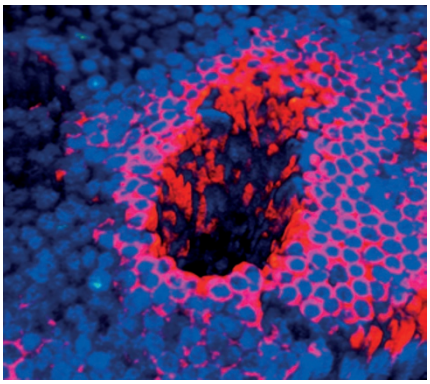
OESOPHAGUS

Widespread self-renewal capacity of human oesophageal epithelial cells



3D reconstruction of human oesophageal epithelium surrounding a papillary area, stained for nuclear marker and cytokeratin 14. Courtesy of R. Fitzgerald & M. Barbera.

Human oesophageal epithelial cells have a remarkable capacity for self-renewal that is not restricted to a specific cell compartment, according to recent findings published in *Gut*.

The human oesophagus is a multistratified squamous epithelium. Traditionally, it has been thought that a distinct set of stem cells is responsible for tissue maintenance, as in other organs of the gastrointestinal tract. However, little is known about the mechanisms underlying cellular homeostasis in the oesophagus. Moreover, studies in mice have failed to demonstrate the presence of a distinct cell set with clonogenic potential.

Rebecca Fitzgerald, corresponding author of the study in *Gut*, and her group have long had an interest in oesophageal carcinogenesis. They joined forces with Phil Jones' group (also based at the Medical Research Council Cancer Unit, Cambridge, UK), who have performed seminal work investigating epithelial self-renewal in normal tissues. "We wanted to shed some light on some fundamental questions, namely: how is the epithelium of the human oesophagus maintained, which cells are responsible for its self-renewal, and is the maintenance of this tissue governed by specific, identifiable stem cells?" explains Fitzgerald. Answers to these questions could potentially contribute to improved understanding of the pathogenesis of Barrett oesophagus and cancers of the oesophagus.

The researchers set out to investigate the patterns of proliferation and mitosis in normal human oesophageal epithelium from 10 patients. The first step was to perform confocal endomicroscopy on epithelial whole mounts. This procedure demonstrated a decrease in the proliferation rate from the interpapillary epithelium to the tip of the papilla. Notably, there was no evidence of cell division at the tip of the papilla. In addition, the orientation of mitosis was randomly distributed throughout the basal layer, and asymmetric divisions (a hallmark of stem cells) were not restricted to any specific cell compartment.

Next, the distribution of three putative epithelial stem cell markers (β1-intergrin, CD34 and MCSP [melanoma chondroitin sulfate proteoglycans]) was investigated—to find out whether different cell lineages might cause this gradient in proliferation along the axis of the papilla. Quiescent cells at the tip of the papilla were found to express each of these markers, suggesting that these cells are epithelial in origin.

To further understand the self-renewal potential of different cell populations, Fitzgerald and co-workers then performed cell sorting according to high and low levels of expression of both an epithelial marker (EpCAM) and stem cell marker (CD34). Thus, four cell groups were identified (EpCAM+CD34+; EpCAM+CD34–; EpCAM–CD34+; and EpCAM–CD34–). These cell populations were then plated into colony-forming cell assays. Interestingly, each of these cell populations had the same or comparable clonogenic potential. This finding persisted even when colonies were grown from single cells.

Finally, the researchers used 3D organotypic models to investigate which cells were likely to be more involved in the maintenance of the tissue. "We made use of the most up-to-date 3D models," explains Fitzgerald. "Previous studies that have used fixed sections for immunostaining can be misleading as it is not possible to gauge exactly where the cells are in 3D space." Isolated cell subfractions of different cell types were able to recapitulate the architecture of squamous epithelium.

Overall, the authors conclude that "despite the distribution of proliferation and stem cell markers, in our model all the cells in the epithelium have the same capacity for clonal expansion, including cells that have already committed to a certain degree of epithelial differentiation and, in theory, should have lost their self-renewing capacity".

Jianwen Que from the University of Rochester, MN, who was not involved in the study, suggests that these findings are important for two reasons: first, cells at different stages of differentiation are proliferative, thus mutations might arise during DNA replication and lead to tumorigenesis; second, cells are more plastic than previously thought and have potential to interchange between different states. He comments, "The immediate questions are: do these cells have similar plasticity *in vivo*, and does the same oncogenic mutation lead to the same outcome (tumour initiation) in these different cell populations?"

Fitzgerald and colleagues believe that their work is extremely important in elucidating mechanisms of tumorigenesis, as vulnerable cells might not be restricted to the so-called 'stem cell' population. They plan to take their work forward using an optimized 3D *in vivo* model for oesophageal epithelium in which they can induce malignant transformation.

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Original article Barbera, M. *et al.* The human squamous oesophagus has widespread capacity for clonal expansion from cells at diverse stages of differentiation. *Gut* doi:10.1136/gutjnl-2013-306171