COLORECTAL CANCER

Zones of biosynthesis

High biosynthetic rates in cells were negatively associated with differentiation.

The pool of cancer stem cells (CSCs) in colorectal cancer (CRC) is continuously regenerated and relays tumorigenic and metastatic capacity in CRC. Yet, CRC lacking cells that express LGR5, a marker commonly used to denote the CSC pool, can maintain primary tumour growth. Thus, LGR5 expression is not sufficient for capturing the entire pool of CSCs. Morral, Stanisavljevic et al. have redefined CRC cell hierarchy based on differential capacity of cells to perform biosynthesis. Undifferentiated CRC cells that have high capacities for ribosomal RNA (rRNA) and protein synthesis were localized immediately adjacent to the stroma, and these capacities were lost upon differentiation.

When analysing patient-derived xenografts (PDXs) from patients with CRC, the authors found that protein biosynthesis, measured by O-propargyl-puromycin (OP-P), which is incorporated into nascent polypeptides, was largely restricted to tumour cells adjacent to the stroma surrounding tumours. Those cells also showed increased synthesis of rRNA as evidenced by measuring incorporation of 5-ethynyl uridine (EU). These CRC biosynthetic zones were enriched with cells expressing ephrin type-B receptor 2 (EPHB2), a marker known to be expressed in

a subset of undifferentiated tumour cells in CRC. Indeed, EPHB2 expression positively correlated with the rates of rRNA and protein synthesis of tumour cells. How was this related to LGR5 expression? Using a CRISPR–Cas9 based knock-in approach, patient-derived tumour organoids (PDOs) were engineered to express a reporter construct for LGR5 expression. PDO-p18, driven by inactivation of *APC* and *TP53*, is known to contain a large number of LGR5⁺ cells, whereas PDO7, driven by mutations in *APC*, *KRAS*, *ATM* and *SMAD4*, contains limited numbers of LGR5⁺ cells. PDO-p18 or PDO7-derived tumour xenografts in mice were then analysed for the location of LGR5⁺ cells, showing that LGR5⁺ cells overlapped with OP-P⁺ tumour domains in PDO-p18 xenografts, whereas LGR5⁺ cells were scattered throughout the tumour in PDO7 xenografts.

High biosynthetic rates in cells were negatively associated with differentiation. The authors showed this in CRC cell lines expressing inducible dominant negative TCF4 and mouse intestinal tumour organoids (tumoroids) expressing *Kras*^{G12D} and a doxycycline (dox)-inducible small hairpin RNA targeting *Apc* and lacking *Trp53* (AKP mouse tumoroids), in which differentiation can be induced through inhibition of Wnt signalling, leading to a reduction of EU and OP-P incorporation. Measuring the protein synthesis rates during cell differentiation by homopropargylglycine (HPG) incorporation showed that as differentiation progressed, nascent protein synthesis progressively declined.

Further analysis identified that cells within the biosynthetic

domains in PDXs were marked by expression of RNA polymerase I subunit A (POLR1A), and expression of POLR1A and the differentiation marker keratin 20 (KRT20) was mutually exclusive. Single-cell RNA sequencing showed that in PDO-p18 xenografts, a large fraction of POLR1A-high cells expressed a gene signature of intestinal stem cells as well as LGR5⁺ cells, whereas there was limited overlap between POLR1A-high and LGR5⁺ cells in PDO7 xenografts. Importantly, lineage tracing analysis suggested that POLR1A⁺ cells represented the main population that fuelled growth of PDO7 tumours, as opposed to LGR5⁺ or KRT20⁺ cells.

Using an inducible caspase 9 knock-in model, the authors analysed whether ablation of POLR1A⁺ or LGR5⁺ cells was affecting tumour growth. When POLR1A⁺ cells were eliminated upon induction of caspase 9, the growth of PDO-p18 or PDO7 xenografts was reduced, whereas ablation of LGR5⁺ cells only reduced growth of PDO-p18 xenografts. Exploring reversibility of the differentiated state in dox-treated AKP mouse tumoroids in vivo, differentiation and reduced EU and HPG incorporation after 8 days of recovery could be rescued by the addition of dox. However, after 20 days of recovery and prolonged differentiation, biosynthetic capacities in CRC cells were permanently downregulated.

Thus the pool of biosynthetic cells in CRC could be reduced for therapeutic benefit, but how this can be achieved pharmacologically needs further study.

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