Trends in Cancer

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Review

Plastic persisters: revival stem cells in colorectal cancer

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Colorectal cancer (CRC) is traditionally considered to be a genetically driven disease. However, nongenetic plasticity has recently emerged as a major driver of tumour initiation, metastasis, and therapy response in CRC. Central to these processes is a recently discovered cell type, the revival colonic stem cell (revCSC). In contrast to traditional proliferative CSCs (proCSCs), revCSCs prioritise survival over propagation. revCSCs play an essential role in primary tumour formation, metastatic dissemination, and nongenetic chemoresistance. Current evidence suggests that CRC tumours leverage intestinal stem cell plasticity to both proliferate (via proCSCs) when unchallenged and survive (via revCSCs) in response to cell-extrinsic pressures. Although revCSCs likely represent a major source of therapeutic failure in CRC, our increasing knowledge of this important stem cell fate provides novel opportunities for therapeutic intervention.

Highlights

Colorectal cancer (CRC) tumours display high levels of nongenetic phenotypic plasticity.

Revival colonic stem cells (revCSCs) are slow-cycling multipotent stem cells found in poor-prognosis CRC tumours.

revCSCs have essential roles in CRC tumour initiation, metastatic dissemination, therapy resistance, and micrometastases.

revCSCs are chemorefractory and can repopulate tumours following therapy.

New knowledge of revCSC plasticity in CRC provides unique opportunities to treat CRC.

Introduction

Colorectal cancer (CRC) (see Glossary) is a carcinoma of the large intestine and rectum that afflicts >900 000 people per year worldwide [1]. CRC tumours are complex heterocellular systems comprising mutated cancer cells, stromal fibroblasts, and multiple immune cells across the **tumour microenvironment (TME)** [2]. Localised early-stage CRC is curable through surgery, but once cancer cells have metastasised, adjuvant drug resistance is common, and there is a desperate need for new therapeutic options.

CRC is widely considered to be a genetically driven cancer. Fundamental observations in 1988 first established CRC as a disease of progressive somatic mutations in *APC*, *KRAS*, and *TP53* [3,4], and contemporary sequencing efforts have further revealed that mutations in *SMAD4*, *PIK3CA*, *TGFBR2*, and *BRAF* are frequently found in CRC tumours [5]. Adding to this genetic perspective, **familial adenomatous polyposis (FAP)** germline *APC* mutations predispose patients to CRC [6], and oncogenic mutations are essential for both CRC metastasis and tumour maintenance [7]. CRC is therefore canonically thought of as a genetically driven cancer, whereby sequential oncogenic mutations confer epithelial stem cells with a fitness advantage over homeostatic epithelia, leading to invasive primary tumours and metastasis.

The healthy colonic epithelium comprises a multipotent basal stem cell compartment that rapidly differentiates into secretory and absorptive cells required for healthy bowel function. **Colonic stem cells (CSCs)** are classically described as LGR5$^+$OLFM4$^+$ and can replenish the murine colonic epithelium in 3–5 days [8]. Cancer cells often retain transcriptomic features of their parental tissue [9], and in CRC, oncogenic mutations polarise epithelia toward a highly mitotic LGR5$^+$OLFM4$^+$ CSC phenotype [10–13]. These exceptionally **proliferative CSCs (proCSCs)** are transcriptionally similar to healthy CSCs, but unlike healthy cells, CRC proCSCs possess hyperactive β-catenin, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and MYC signalling and high signalling entropy [13]. Moreover, unlike healthy CSCs, oncogenic proCSCs

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are trapped in a mitotic state and fail to differentiate into the secretory and absorptive cells of the healthy colon [13].

Collectively, these observations support a genetically dominant model of CRC where oncogenic mutations cell-autonomously dysregulate cancer cell signalling, turning all cancer cells into hyperproliferative stem-like cells. However, despite the importance of somatic mutations in driving CRC, evidence of nongenetic **plasticity** has recently been shown to underpin metastasis, immune evasion, and therapy resistance across multiple cancer types [14,15]. This is especially true for CRC, where, despite common genetic drivers, several studies have demonstrated surprising phenotypic plasticity in CRC tumours. For example, depletion of LGR5$^+$ cancer cells can arrest primary CRC development, but isogenic LGR5$^-$ cells can dedifferentiate back into LGR5$^+$ cells to reform tumours [16]. Moreover, transcriptional heterogeneity between CRC patient tumours cannot be explained by underlying genetic alterations, suggesting that nongenetic processes regulate phenotypic diversity [17]. These observations imply that although oncogenic mutations are clearly required for CRC formation and maintenance, phenotypic plasticity is common in CRC and can occur via nongenetic mechanisms. This review will focus on a major emerging source of nongenetic plasticity in CRC, revival stem cells.

Revival stem cells

The healthy intestinal epithelium separates the nutrients, waste, and microbial contents of the distal gastrointestinal lumen from the host cells of the lamina propria. Like other barrier tissues, such as the skin or lung, intestinal epithelia must rapidly regenerate to restore barrier function following injury or infection. Given the rapid turnover of intestinal epithelia under homeostatic conditions, one might presume that traditional LRG5$^+$ intestinal stem cells would quickly regenerate epithelial integrity following injury. However, early mouse model studies revealed that LGR5$^+$ stem cells are dispensable in the homeostatic small intestine [18], and slow-cycling stem cells adjacent to the traditional crypt base (in the +4 position) can give rise to all intestinal lineages [19]. These results were the first indication that intestinal stem cells are highly plastic, with cells outside the traditional LGR5$^+$ stem cell pool able to repopulate epithelia following injury.

A series of seminal papers in 2018–2019 subsequently revealed that a new epithelial cell type was responsible for intestinal regeneration. It was first shown that, following colonic injury, the epithelia became enriched for a new type of stem cell that was not present in the homeostatic adult intestine [20]. Similar stem cells were also found following *Heligmosomoides polygyrus* infection [21] and intestinal irradiation [22]. These new stem cells all bore transcriptional similarity to **foetal intestinal stem cells** [23,24], suggesting that the intestine engages early developmental programmes in response to epithelial stress. Given their ability to repopulate the entire intestinal epithelium following injury, these stem cells were named revival stem cells [revSCs; or **revival CSCs (revCSCs)** in the colon].

Unlike classical proliferative LGR5$^+$ stem cells, revSCs are LGR5$^-$ and slow-cycling. revSCs express *Anxa1*, *Clu*, and *Sca1/Ly6A* and are characterised by high **Yes-associated protein (YAP)** signalling [20,22,24,25]. revSCs have also been found to possess high FAK [20], tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interferon gamma (INF-γ) [26], and NF-κB signalling [24] and evidence of autophagy [27]. The Hippo pathway is a major regulator of intestinal regeneration and symmetry breaking [28], and revSCs appear to leverage YAP signalling to support epithelial regeneration. Notably, high YAP activity also correlates with increased TNF-α, TGF-β, INF-γ, and NF-κB signalling across a range of tumours [29], indicating that revSCs may invoke a fundamental inflammatory signalling state during intestinal regeneration. High YAP activity is inversely proportional to classical LGR5$^+$ stem cells [28] and

Glossary

Cancer-associated fibroblasts (CAFs): mesenchymal stromal cells found in the tumour microenvironment that secrete growth factors and extracellular matrix into the tumour microenvironment and correlate with poor prognosis in CRC.
Colonic stem cells (CSCs): multipotent epithelial stem cells found in the homeostatic colon that can differentiate into all colonic epithelial cells.
Colorectal cancer (CRC): epithelial carcinoma (typically an adenocarcinoma) of the large intestine and rectum.
Consensus molecular subtypes (CMSs): a CRC classification system that resolves tumours into CMS1–CMS4 based on bulk transcriptomic signatures.
Drug-tolerant persister: a cancer cell that can survive anticancer therapy that is often responsible for disease relapse.
Familial adenomatous polyposis (FAP): an inherited condition caused by germline mutations in the *APC* gene resulting in numerous epithelial polyps in the colon that predisposes patients to CRC.
Foetal intestinal stem cells: intestinal stem cells from the developing foetus that are transcriptionally distinct from adult intestinal stem cells but resemble revCSCs with high levels of YAP signalling.
Revival CSCs (revCSCs): multipotent colonic stem cells that are enriched during tissue damage and can regenerate all cells of the colonic epithelium. revCSCs are frequently found in poor-prognosis CRC and are characterised by low β-catenin and PI3K signalling and high YAP signalling.
Patient-derived organoid (PDO): biomimetic 3D *in vitro* organoid model of an individual patient's cancer cells that can be used to study personalised tumour biology and drug responses.
Plasticity: the ability of a cell to change fate. Cells with high plasticity can easily change fate, whereas cells with low plasticity struggle to change fate.
Prostaglandin E_2 (PGE_2): eicosanoid prostaglandin synthesised by phospholipase A2 and COX enzymes. PGE_2 can be secreted by colonic fibroblasts to activate epithelial YAP signalling.
Proliferative CSCs (proCSCs): a hyperproliferative colonic stem cell found in CRC that rarely differentiates into other colonic epithelial cells. proCSCs are characterised by high β-catenin, MAPK,

polarises intestinal organoids toward a cyst-like growth-stalled state [20,30,31]. Despite their quiescence, revSCs act as a clonogenic reserve that can later revert to a proliferative state capable of differentiating into all epithelial cell types [32]. Collectively, unlike classical β-catenin-driven LGR5^{+} stem cells, revSCs appear to use Hippo and NF-κB signalling to enter a slow-cycling, yet multipotent, state that enables intestinal epithelia to survive extreme cellular stress and repopulate the epithelium following tissue damage.

The acute enrichment of revSCs during intestinal injury implies that CSCs and differentiated epithelia can rapidly polarise to revSCs. High-dimensional phenoscaping [33,34] of CSC polarisation revealed that when epithelial cells experience high MAPK, PI3K, and β-catenin signalling [via oncogenic mutations or stromal R-spondin or epidermal growth factor (EGF) ligands], cells polarise to proCSCs [13]. By contrast, when cells experience low MAPK and PI3K signalling and high YAP activity (via stromal WNT-3A or TGF-β), epithelia differentiate to revCSCs. These observations revealed that epithelial stem cells exist on an interconnected stem cell trajectory that can be rapidly traversed in response to stromal cues and oncogenic mutations (Figure 1).

and PI3K signalling and low YAP signalling.

Tumour microenvironment (TME): stromal and immune cells that surround cancer cells in tumours.

Yes-associated protein (YAP): a transcriptional coregulator of the Hippo signalling pathway that is upregulated in revCSCs.

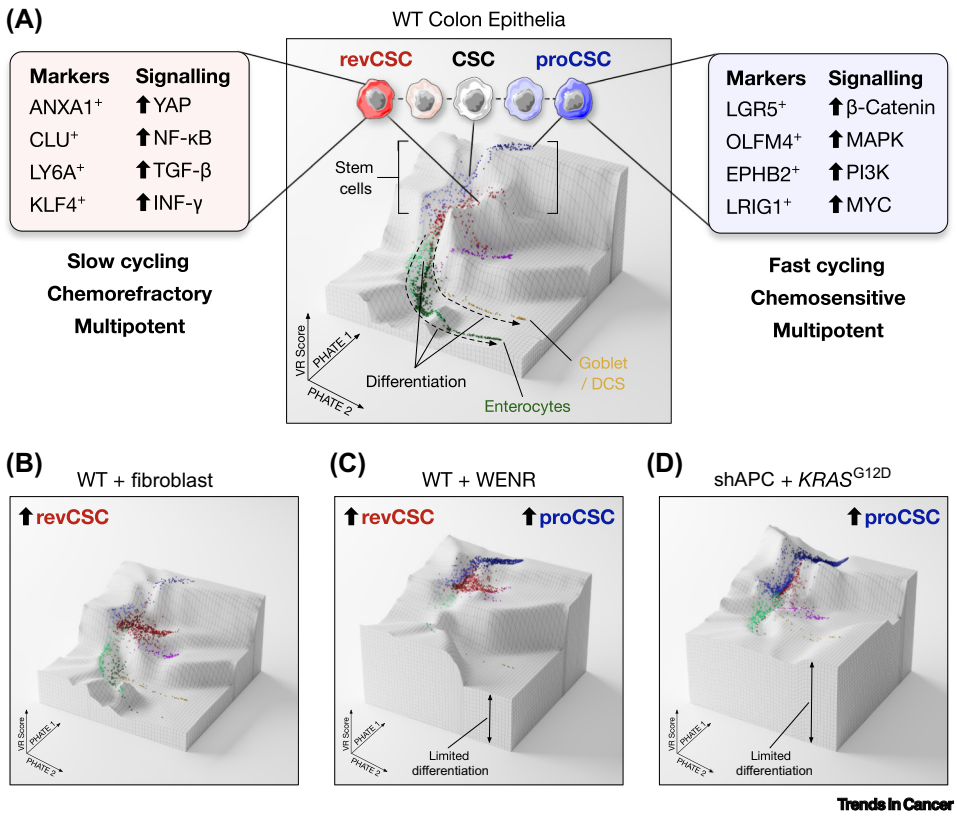

Figure 1. Intestinal epithelial stem cell plasticity. (A) Single-cell RNA-sequencing (scRNA-seq) valley-ridge (VR) landscape of wild-type (WT) epithelial colonic organoids [13] illustrating proliferative colonic stem cell (proCSC; blue)-to-revival colonic stem cell (revCSC; red) trajectory, transit amplifying cells (pink), endoplasmic reticulum (ER) stress (magenta), goblet/deep crypt secretory (DCS) cells (yellow), and early/late enterocytes (light/dark green). (B) WT organoids and colonic fibroblasts polarise to revCSCs. (C) WT organoids grown in WNT-3A, epidermal growth factor (EGF), Noggin, and R-spondin-1 (WENR) polarise to revCSCs and proCSCs, with limited differentiated cells. (D) Colorectal cancer (CRC) organoids with oncogenic shAPC and *KRAS*^{G12D} are polarised to proCSCs, with limited differentiation. Abbreviations: INF, interferon; PI3K, phosphoinositide 3-kinase; TGF, transforming growth factor.

Revival stem cells in CRC

Cancer occurs when cellular fitness becomes dominant over organismal fitness. To achieve increased cellular fitness, cancers often hijack existing developmental programmes to explore phenotypic space beyond homeostatic constraints. Given their powerful role in regenerating damaged epithelia, it is unsurprising that CRC also leverages revCSC programmes throughout oncogenesis, metastasis, and therapy response.

Although LGR5^{+} stem cells are often considered the cell of origin for CRC, YAP signalling is required for adenoma formation in *Apc*$^{-/-}$ mice, suggesting a possible role for revCSCs in early tumour formation [25]. In agreement with this, stromal-derived **prostaglandin E_2 (PGE_2)** can activate epithelial YAP, driving revCSCs to support early CRC tumour formation [35]. These results strongly imply that revCSCs are important in CRC tumour initiation. However, YAP overexpression inhibits β-catenin signalling (via blocked DVL translocation [36] and/or binding of YAP to TLE [37]), which can in turn downregulate proliferative LGR5^{+}OLFM4^{+} stem cells [36] and supress the growth of CRC tumours [38]. These contradictory findings have even led to YAP being labelled as a tumour suppressor in CRC [38] despite the fact that high YAP activity correlates with poor prognosis [29]. Collectively, these results imply that YAP-driven revCSCs are involved in tumour initiation, but YAP signalling alone depletes proliferative stem cells and slows tumour growth. As a result, the exact role of revCSCs in CRC was initially unclear.

While revCSCs are clearly important for early tumour formation, they cannot expand to form primary tumours due to their slow cell-cycle activity [38]. Neoplasia requires proliferation, and mitotic LGR5^{+} cells are therefore necessary to establish primary tumours [39]. However, once established, primary human CRC tumours are rarely dominated by just proCSCs and instead are composed of an admixture of LGR5^{+}OLFM4^{+} proCSC and ANXA1^{+} revCSC cancer cells [26]. These findings imply that CRC balances the complimentary phenotypes of proCSCs and revCSCs to establish primary tumours.

Although one might expect tumours dominated by highly proliferative proCSCs to underpin poor prognosis, an abundance of slow-cycling revCSCs predicts worse survival in multiple human CRC cohorts [40]. This counterintuitive observation is a reminder that the lethality of cancer is dependent on more than just hyperproliferation. CRC tumours can be broadly categorised into four **consensus molecular subtypes (CMSs)** [41]. Of these, CMS2 tumours are dominated by epithelial cells and are often polarised toward proCSC-like cells [13,26]. By contrast highly stromal CMS4 tumours have increased revCSCs [13,26]. These stromal revCSC-dominant tumours have a poor response to chemotherapy and poor prognosis [42,43], suggesting that revCSCs may be a major source of therapy resistance in CRC.

Stem cell plasticity in CRC chemotherapy response

Before the plasticity of CRC cancer cells was fully appreciated, early studies in cell lines demonstrated that 5-fluorouracil (5-FU) chemotherapy can induce a slow-cycling cell state in CRC [44]. CRC liver metastases *in vivo* were also found to express higher levels of YAP following neoadjuvant 5-FU chemotherapy, and high YAP expression positively correlated with disease relapse and shorter patient survival [44]. LGR5^{+} cells can also enter a slow-cycling state via PROX1 to resist chemotherapy [45]. Collectively, these results hinted that dynamic cell fate transitions could underpin CRC chemotherapy response, but the exact nature of this plasticity was unclear. It was recently confirmed that low-dose chemotherapy induces not just YAP expression but a full revCSC fate switch in **patient-derived organoids (PDOs)** [40]. This phenomenon has been observed in multiple human PDOs, including the identification of MEX3A^{+} revCSCs that are chemoresistant and can also repopulate CRC tumours following chemotherapy [46]. Eloquent

lineage tracing models have further revealed that, in theory, any cell within a CRC tumour can enter a transient slow-cycling, **drug-tolerant persister** state during chemotherapy treatment and that persister cells can repopulate tumours after treatment [27]. The finding that any isogenic cancer cell can achieve drug tolerance underscores the fundamental importance of nongenetic plasticity in CRC.

Collectively, our current understanding of CRC therapy response implies that CSC plasticity permits a 'chemorefractory plasticity cycle' in both primary and metastatic CRC (Figure 2). Under this model, established tumours comprise an admixture of proCSC and revCSC cancer cells. Following chemotherapy, highly proliferative proCSCs are killed in the initial response phase, but slow-cycling revCSCs can resist antimitotic therapies. revCSCs are tolerant to prolonged chemotherapy and persist as residual disease. Once treatment is stopped, revCSCs can repolarise to proCSCs, rapidly proliferate, and replenish tumours. Relapsed tumours can then reestablish the proCSC–revCSC admixture, enabling CRC tumours to withstand future rounds of systemic chemotherapy.

Stem cell plasticity in CRC metastasis

Although surgical resection of primary early-stage CRC tumours is often curative, nodal and distant metastases are responsible for residual disease and ultimately patient death. It is therefore crucial that we understand the processes required for CRC metastasis to prevent and treat late-stage CRC. Seminal work across mouse models and human samples has now demonstrated that stem cell plasticity is a central component of CRC metastasis.

The first indication that plasticity was important for metastasis was the observation that disseminating CRC cells are LGR5^{-} but revert to LGR5^{+} once metastatic lesions are established [47]. This finding was then confirmed in human samples whereby early CRC micrometastases are devoid of proliferative OLFM4^{+} stem cells but enriched for YAP gene expression signatures [30]. Metastasis-initiating cells have also been shown to express L1CAM, have an overlapping gene signature with revCSCs [48], and possess active YAP signalling [49]. These results imply that early disseminating CRC cells are revCSCs. However, given that YAP signalling inhibits proliferation, micrometastatic CRC cells must switch from revCSCs to proCSCs to expand into larger macrometastases [30]. In addition to promoting survival, metastatic revCSCs can further differentiate into noncanonical squamous and neuroendocrine-like states (via PROX1) that correlate with poor outcome [50].

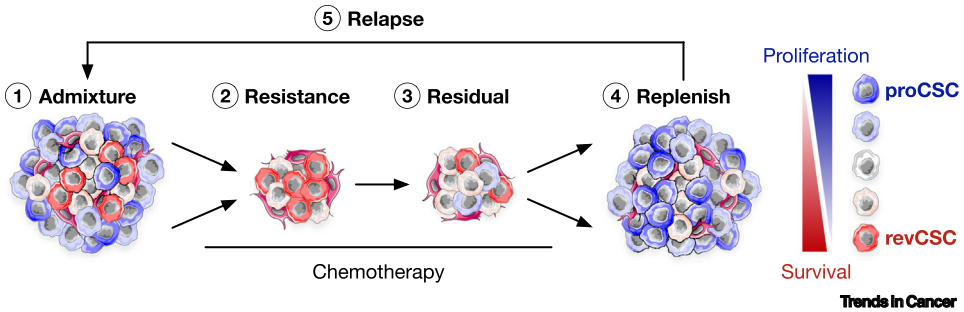

Figure 2. Colorectal cancer (CRC) chemorefractory plasticity cycle model. (1) CRC tumours comprise an admixture of proliferative colonic stem cells (proCSCs) and revival colonic stem cells (revCSCs). (2) Chemotherapy eliminates mitotic proCSCs and enriches a slow-cycling revCSC polarised resistant population. (3) Residual disease can withstand chemotherapy in a revCSC-dominant state. (4) Once treatment is complete, CRC cells can polarise to proCSCs to replenish tumours. (5) Relapsed CRC tumours can restore the proCSC–revCSC admixture.

Cells driving metastatic relapse following primary tumour resection are also LGR5^{-} and EMP1^{+} and slow-cycling [51]. These high-relapse cells are ANXA1^{+} and show overlap with revCSC gene signatures [13] but are not dependant on YAP signalling and may represent an intermediate cell state between proCSCs and revCSCs and/or squamous-like differentiation [50]. Importantly, genetic depletion of EMP1^{+} cells can protect mice against metastatic relapse, highlighting the functional importance of revCSC-like LGR5^{-} cells in CRC prognosis. Interestingly, revCSC-like micrometastases are highly enriched for immune infiltrates and can even be eradicated using immune checkpoint inhibitor therapies [51]. This last observation serves as a reminder that although cellular plasticity complicates traditional cancer therapies, our increasing knowledge of cancer cell fate transitions also opens new opportunities for therapeutic intervention.

Collectively, these results suggest that stem cell plasticity is a fundamental feature of metastatic CRC (Figure 3). The current data support a model whereby early disseminated tumour cells leverage revCSC programmes to survive in circulation and seed micrometastases. These early metastatic revCSCs are slow-cycling and therefore inherently chemorefractory. Once established at a metastatic site, cancer cells switch from slow-cycling revCSCs to a proliferative proCSC-like fate, expanding to form macrometastases. These metastatic proCSCs are chemosensitive but highly plastic and can escape adjuvant chemotherapy by transiently reverting back to revCSCs upon treatment via the 'chemorefractory plasticity cycle' described earlier (Figure 2) or by differentiating into noncanonical cell types (e.g., squamous and neuroendocrine cells). Following treatment withdrawal, drug-tolerant revCSCs can then switch back to proCSCs to repopulate tumours.

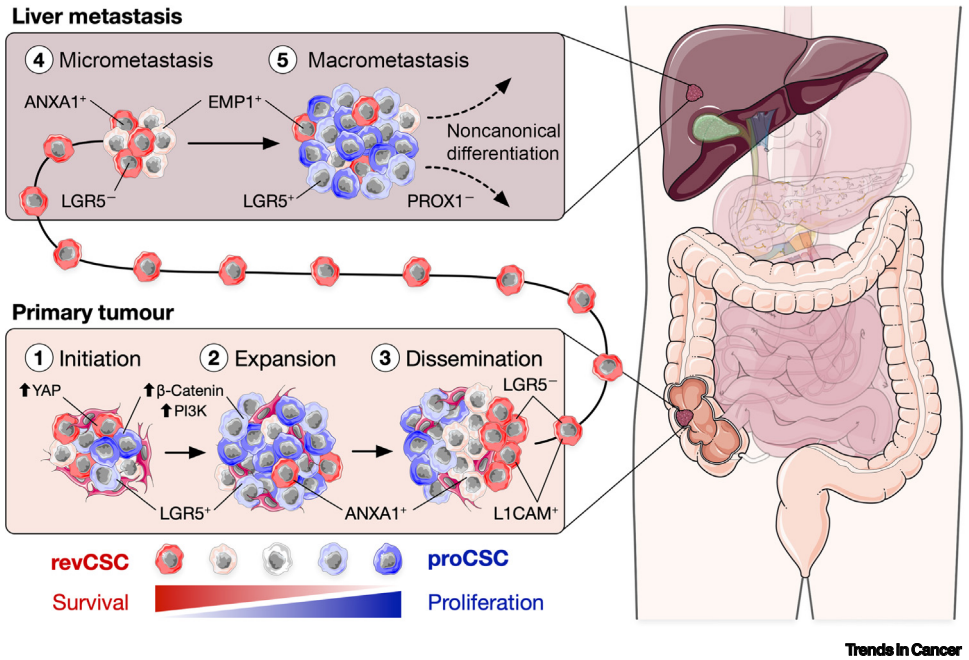

Figure 3. Plasticity in colorectal cancer (CRC) metastasis. (1) Primary tumours can initiate via revival colonic stem cells (revCSCs). (2) Tumours expand via proliferative colonic stem cells (proCSCs) and ultimately comprise an admixture of stem cells. (3) Disseminated tumour cells polarise toward revCSCs to survive circulation. (4) CRC cells retain a revCSC phenotype to establish micrometastasis. (5) Once established, revCSCs can switch back to proCSCs to expand into macrometastasis or differentiate into noncanonical cell types. Abbreviations: PI3K, phosphoinositide 3-kinase; YAP, Yes-associated protein.

CRC stem cell plasticity therefore provides an 'advance and cover' mechanism of chemoprotection. When either the primary tumour or metastatic site expands, CRC cells enter a proliferative proCSC state and 'advance' When under attack, CRC cells 'cover' by switching to revCSCs, slowing their cell cycle and surviving chemotherapy. By rapidly cycling between 'advance' and 'cover' states, stem cell plasticity enables CRC tumours to both proliferate and resist antiproliferative therapies.

Regulation of CSC plasticity

CRC leverages CSC plasticity to both proliferate when unchallenged (proCSCs) and survive when stressed (revCSCs). Given that CRC tumours comprise an isogenic admixture of proCSCs and revCSCs *in vivo* [26], and proCSC cancer cells can rapidly polarise to revCSCs following therapy [40], one would expect acute, reversable, nongenetic processes to regulate CSC polarisation, namely protein-level signalling.

In wild-type colonic epithelia, revCSC polarisation can be driven by stromal TGF-β and/or WNT-3A activation of YAP [13,20]. However, the route to revCSCs in mutated CRC cells is less clear and will likely vary from patient to patient [50,52] and involve epistatic effects [53]. For example, when colonic epithelia have either lost APC or express oncogenic $KRAS^{G12D}$, stromal TGF-β and WNT-3A can still induce revCSCs. However, when the epithelia have lost APC and express $KRAS^{G12D}$ (as often found in CRC), epithelia become polarised to proCSCs and downregulate homoeostatic signalling receptors [13]. Under this strong oncogenic signalling load, stromal TGF-β and WNT-3A cannot induce revCSCs [13]. This implies that signalling downstream of oncogenic mutations is inherently biased toward proCSCs and limits access to revCSCs. However, if PI3K signalling is inhibited in CRC cells, stromal TGF-β can polarise proCSC cancer cells to revCSCs despite upstream oncogenic mutations. Moreover, TGF-β can also induce revCSCs via YAP in CRC organoids containing oncogenic *KRAS/BRAF* and loss of *TP53* and *SMAD4* [35]. These results suggest that accessing revCSCs in CRC is dependent on parallel post-translational modification (PTM) signalling downstream of APC, KRAS/BRAF, and TGF-β. Simply activating the YAP pathway is not enough; CRC cells must also have low MAPK/PI3K and/or β-catenin signalling activity to transition from proCSCs to revCSCs.

Stromal-rich CMS4 tumours have elevated revCSC cancer cells [26] and a worse prognosis [42,43]. Moreover, epithelial YAP signalling can be activated by fibroblast-derived PGE_2 [35] and high extra cellular matrix (ECM) stiffness [54], and stromal TGF-β can directly induce revCSCs [13]. As a result, one might expect **cancer-associated fibroblasts (CAFs)** to regulate revCSC abundance in CRC tumours. Analysis of PTM signalling, cell cycle activity, DNA damage, and apoptosis in >2500 PDO-CAF cocultures during therapy response revealed that CAFs can induce revCSC formation in a patient-specific manner [52]. While proCSC PDOs alone are highly sensitive to irinotecan, 5-FU, and oxaliplatin, CAF-induced revCSC cancer cells are resistant to all chemotherapies. CAF-induced proCSC-to-revCSC polarisation can occur within hours of coculture and is rapidly reversable following CAF removal, suggesting that the proCSC-to-revCSC transition is driven by acute PTM signalling (via YAP and NF-κB). Interestingly, CAF-induced revCSCs still experience on-target cell cycle blockage and DNA damage following chemotherapy treatment, but drug-induced genotoxic stress does not lead to apoptosis in revCSCs. This further implies that CAFs are a major driver of revCSC polarisation and that increased access to revCSCs could underpin chemoresistance in highly stromal tumours (Figure 4A). Much like traditional proCSCs, CAF-induced revCSC cancer cells retain high signalling entropy [52] (indicative of multipotency [55]), but unlike proCSCs, revCSCs are slow-cycling, enabling them to escape the on-target effects of chemotherapy. Collectively, these results suggest that CAF-induced revCSCs are an optimal drug-tolerant persister cell that are chemoresistant with high regenerative capacity.

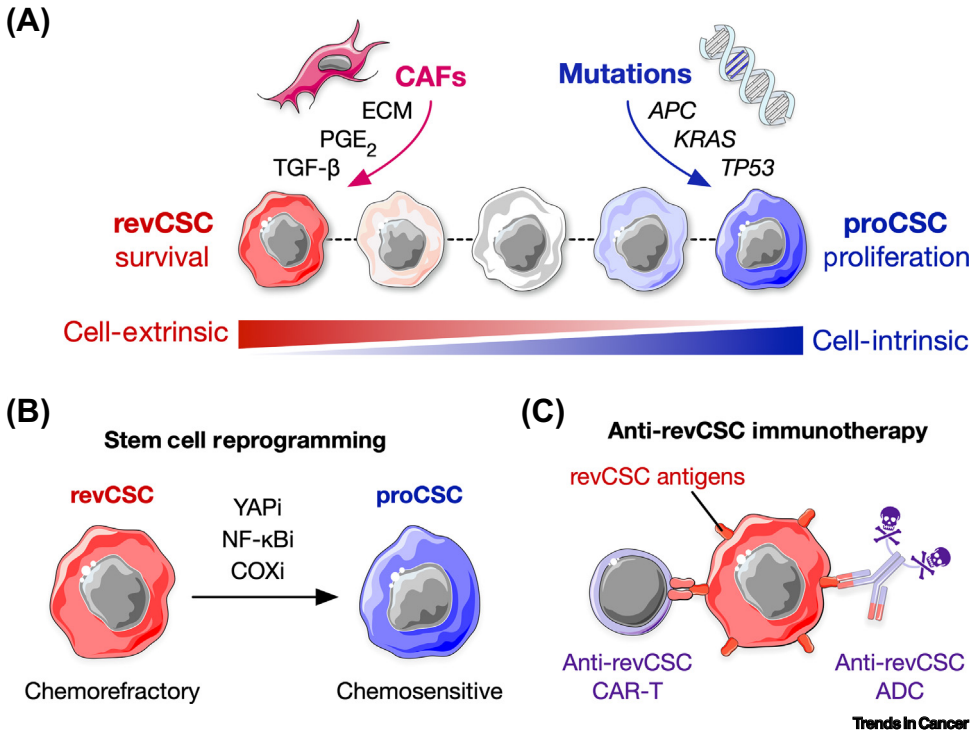

Figure 4. Colonic stem cell (CSC) regulation and therapeutic opportunities (A) Regulators of the proliferative CSC-to-revival CSC (proCSC-to-revCSC) axis. proCSCs are driven by cell-intrinsic colorectal cancer (CRC) oncogenic mutations, whereas revCSCs are often regulated by microenvironmental cues. (B) Pharmacological reprogramming of revCSCs back to proCSCs could resensitise CRC cells to chemotherapy. (C) As revCSCs are rare in the homeostatic colon, revCSC-specific antigens could provide excellent targets for chimeric antigen receptor T cell (CAR-T) and antibody–drug conjugate (ADC) immunotherapies. Abbreviations: CAF, cancer associated fibroblast; ECM, extra cellular matrix; PGE_2, prostaglandin E2; TGF-β, transforming growth factor beta.

Concluding remarks

Since the original identification of germline and somatic driver mutations, CRC has long been considered to be a genetically driven disease. However, recent studies have revealed that non-genetic plasticity is also a major driver of CRC tumour initiation, maintenance, metastasis, and therapy resistance. In CRC, plasticity often leverages a foetal-like revival stem cell population that has complimentary features to traditional fast-cycling intestinal stem cells. Access to such early developmental programmes could be a common feature of cancer plasticity, as cellular reprogramming often requires a transition through an early diapause-like state [56]. Beyond CRC, other cancers leverage slow-cycling phenotypes to survive antitumour challenges (including immunosurveillance [57]), suggesting that the 'advance' and 'cover' model described by proCSCs and revCSCs could be more broadly applicable.

While phenotypic plasticity enables cancer cells to evade traditional chemotherapies, our new appreciation of the proCSC–revCSC transition in CRC provides opportunities for novel therapeutic interventions (see Outstanding questions). Given their crucial role in drug tolerance and disease relapse, future efforts to directly eliminate revCSCs in CRC are now essential (Figure 4B). Due to their slow-cycling nature, revCSCs are inherently chemorefractory and therefore cannot be targeted using existing antimitotic therapies. In fact, small-molecule inhibition of mitotic signalling pathways (e.g., PI3K and MAPK) may actually increase revCSCs and contribute to chemotherapy failure [13]. Instead, revCSCs are dependent on antiproliferative Hippo signalling. It is currently

Outstanding questions

Several stromal cues have been shown to induce revCSCs (e.g., TGF-β, WNT-3A, and PGE_2). How are revCSCs regulated in human tumours and how does this vary from patient to patient?

Can signalling networks (e.g., YAP and NF-κB) or antigens unique to revCSCs be directly targeted via small molecules, immunotherapies, or cellular therapies to treat CRC?

If revCSCs represent a transient plasticity state, can revCSCs be converted back into proCSCs to resensitise cancer cells to existing chemotherapies?

What is the role of revCSCs in MSI CRC and how do revCSCs influence immunotherapy responses?

Nongenetic plasticity has emerged as a hallmark of CRC. What other cell states are important for disease progression?

unclear if YAP–TEAD inhibition is clinically actionable in CRC, but evidence that chemorefractory revCSCs can be resensitised to chemotherapy via YAP inhibition provides incentive to explore this concept further [52]. A new generation of YAP–TEAD inhibitors is currently entering the clinic [58], and the application of these inhibitors in CRC as stem cell-reprogramming agents should be explored. Future efforts to target CAF-induced revCSCs via inhibiting TGF-β, WNT-3A, and PGE_2 signalling are also ongoing. While inhibiting revCSC pathways provides one avenue, an alternative approach to resensitise revCSCs to chemotherapy could be to reactivate mitotic signalling pathways in slow-cycling cancer cells before chemotherapy treatments. For example, chemosensitive proCSCs are dependent on PI3K signalling, whereas chemorefractory revCSCs have very low PI3K activity [13]. Recent advances in kinase activator drugs now enable transient therapeutic activation of PI3K [59] that could force slow-cycling stem cells to re-enter the cell cycle and become chemosensitive. Future efforts to reprogramme cellular plasticity should therefore consider signalling activation as well as inhibition.

revCSCs are rare in the homeostatic intestine, and thus revCSC-specific antigens could be an excellent target for chimeric antigen receptor T (CAR-T) cell therapies or antibody drug conjugates (ADC) (Figure 4C). Neoadjuvant immune checkpoint blockage therapy is revolutionising the treatment of microsatellite instability (MSI) CRC but has so far had limited impact on microsatellite stable (MSS) CRC [60]. However, mouse models of MSS CRC micrometastases suggest that $EMP1^+$ revCSC-like cells might also be targetable by anti-programmed cell death protein 1 (PD-1) antibodies [51]. It is currently unclear if revCSCs are generally more sensitive to immune checkpoint blockade (as they will likely contain an identical tumour mutational burden to isogenic proCSCs of the same tumour), but future efforts to eliminate revCSCs via immunotherapies should be explored.

Despite the exciting opportunities provided by targeting revCSCs clinically, much of our current understanding of CSC plasticity is derived from defined mouse models that do not recapitulate the complexity of human CRC. The mechanisms regulating proCSC-to-revCSC polarisation will likely vary from patient-to-patient [50,52], and future studies will need to explore why some patient tumours comprise high revCSC admixtures and why some cancer cells can rapidly adopt revCSCs and others do not [26]. This interpatient stem cell heterogeneity will likely not be explained by underlying genetic changes [17], so alternative molecular modalities (e.g., mRNA, epigenetics, PTM signalling, and metabolites) should be explored in large patient cohorts.

Finally, although this review has focused on the pathological importance of revCSCs in tumour initiation, metastasis, and drug resistance, intestinal epithelia are highly dynamic, and dedifferentiation beyond the stem cell pool can also facilitate cancer plasticity. For example, anti-EGF therapies can induce a Paneth-like cell fate transition that underpins therapy resistance [61]. These findings serve as a reminder that phenotypic plasticity is diverse and that fates beyond the proCSC-to-revCSC trajectory are also likely to play an important role in CRC progression.

Collectively our new appreciation of the intestinal stem cell landscape has established nongenetic plasticity as a hallmark of colonic epithelia. Early insights into intestinal regeneration following injury have laid the foundation for stem cell plasticity concepts, which have since become central to our mechanistic understanding of CRC initiation, outgrowth, and metastasis. Our current model suggests that stem cell plasticity affords CRC cells the complimentary options to both proliferate when unchallenged (proCSCs) and survive when stressed (revCSCs). This model may explain why phenotypic variance cannot be clearly understood by genotype alone and provides a mechanism for systemic therapy failure in highly stromal tumours. While plasticity remains a formidable challenge in cancer therapy, the identification of unique cell types now provides a

novel therapeutic opportunity to target these cells. Future efforts to eliminate revCSCs are ongoing and will represent a new frontier in CRC therapy.

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Declaration of interests

The author declares no competing interests.

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