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REVIEW

The cancer cells-of-origin in the gastrointestinal tract: progenitors revisited

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Abstract

A prominent model of tumor progression posits that normal self-renewing and multipotent stem cells (SCs) are the initial target of transformation. This view has been robustly challenged by the recurring observation that transit-amplifying cells and differentiated progenitors can initiate neoplasia outside the SC zone thus qualifying as cancer cells-of-origin. The emerging concept is that a cancer SC and a cancer cell-of-origin are not necessarily the same cell. Importantly, progenitor cells were shown to possess remarkable plasticity and to revert, on demand, to a SC-like state. The present review revisits our early hypothesis that colonic progenitors acquiring a mutant adenomatous polyposis coli gene after exiting the stem zone may serve as genuine cancer cells-of-origin. New findings consonant with this view are examined, and tenable molecular and cellular mechanisms underpinning the plasticity of progenitor cells in the gastrointestinal tract and in other tissues are discussed. The translational impact of cell plasticity is addressed, and recommendations for future research are advanced.

Introduction

A prominent model of tumor progression posits that normal self-renewing and multipotent stem cells (SCs) are the initial target for transformation (1). In this view, a normal SC would acquire a heritable change, an oncogenic-driven mutation that originates a mutant clone. The rogue clone would then spawn a larger, early preneoplastic population that, after acquiring an advantageous genetic hit, will directly generate the next mutant SC population. This process of Darwinian selection continues until the final cancer stem cell (CSC) population present in a fully fledged tumor. Implicit in this unidirectional model is the notion that a mutant SC subpopulation evolves directly into the next mutant SC subpopulation. The rest of tumor derived from CSCs, which contributes to tumor heterogeneity, consists of phenotypically diverse cells with limited proliferative capacity and feeble tumorigenic potential. In this scenario, progenitor cells in the colonic crypt fail to qualify either as cancer cells-of-origin or—of—propagation. The residence of Lgr 5^+ SCs in the colonic crypt base (2,3) has been frequently cited as robust evidence for the 'bottom-up model' of adenoma histogenesis (4,5), which posits that a mutant SC clone with a growth advantage would expand from the stem zone to fill an entire colonic crypt (Figure 1B).

The colonic crypt: a hub of biological events

It is pertinent at this point to expound briefly on the functional definition and dynamics of colonic cells once they leave the crypt stem zone and migrate towards the crypt surface (Figure 1A). The colonic crypt is a veritable hub of biological events. The immediate SC progeny cells after exiting the stem crypt zone undergo a finite round of divisions in the mid-crypt compartment to expand the cell pool available for final lineage commitment; consequently, these rapidly proliferating cells are referred to as transit-amplifying (TA) cells. Moving on orderly towards the crypt cuff, TA cells withdraw from the cell cycle, enter the crypt post-mitotic compartment, and commence the differentiation program to generate all colonic crypt cell lineages (6). Notably, two major molecular events occurring in a progenitor cell entering the differentiation compartment of the crypt are the progressive expression

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Abbreviations

APC	adenomatous polyposis coli
CSC	cancer stem cell
SC	stem cell
TA	transit-amplifying

of the gatekeeper tumor suppressor gene adenomatous polyposis coli (APC in humans, *Apc* in mice) and the concomitant silencing of the Wnt/β-catenin/TCF4 signaling pathway (7). The Wnt transduction pathway is markedly active in the stem zone and in the lower proliferative compartment of the colonic crypt (7,8); the APC protein is part of a multiprotein complex involved in downregulation of Wnt activity. Consequently, APC and the Wnt pathway exhibit opposite expression gradients along the colonic crypt continuum (7), (Figure 1A).

The journey of the progenitor cells towards the colonic crypt cuff is completed within 4–5 days. Three main differentiated epithelial cell lineages populate the colonic crypt: the absorptive columnar cells, or colonocytes, the secretory goblet cells and the endocrine cells producing and secreting peptide hormones. An additional epithelial secretory cell type, dubbed 'tuft cell', has been recognized (9). A subset of colonic goblet cells at the bottom of the crypt appears to be functionally similar to the Paneth cells of the small intestine (10). Once at the crypt luminal surface, the terminally differentiated adult cells, after performing for a short period of time their specialized functions, die by apoptosis or are forcibly excluded; ultimately, the doomed cells are shed into the colonic lumen. Stringent maintenance of cellular census and tissue homeostasis are assured by the steady supply of new crypt cell populations fuelled by SCs rooted to the crypt bottom: in the colonic crypt, cell demise is exquisitely coupled to cell renewal.

Gastrointestinal progenitors: cancer cells-of-origin

Some years ago, Lamprecht and Lipkin (11) advanced the hypothesis that a colonic TA daughter cell acquiring a mutant APC gene would qualify as a cancer cell-of-origin (Figure 1C). APC was selected as the gene bearing the first mutational hit in a progenitor cell since mutations in APC gene are the earliest found in 80% of colonic adenomas (12). It was suggested that additional mutations (e.g. K-Ras, TGF-β) and epigenetic changes would allow the mutant clone to acquire a more aggressive phenotype and, in time, to grow into an adenoma. This model is consistent with the finding that early adenomatous polyps are frequently detected at the top of colonic crypts (13) without an evident connection with the SC zone, suggesting a 'top-down' model of adenoma morphogenesis that proposes that dysplasia originates in the colonic luminal surface and spreads downwards (13,14), (Figure 1C). The expression gradient of the APC protein along the normal colonic crypt (Figure 1A) is consistent with this model.

The main proposition of our model is that a non-stem progenitor cell could serve as a cancer cell-of-origin. A frequent criticism leveled at this hypothesis strongly relies on the assumption that a short-lived mutant progenitor clone would be washed out into the colonic lumen and invariably lost as a putative cancer-initiating cell. Surprisingly, this view fails to take into consideration the well-recognized involvement of wild-type APC not only in restraining the activity of Wnt pathway, but also in controlling the ordered ascent of normal TA cells and differentiating progenitors along the colonic crypt continuum (15–18). Indeed, one immediate consequence of *Apc* loss in murine epithelium *in vivo* was shown to be a marked perturbation of cell migration, and *Apc*-deficient mice were defined as maintaining 'a crypt-progenitor like phenotype' (16). It is worth noticing that defects in intestinal migration and abnormal cell accumulation were noted in mouse *Apc* heterozygotes (17), an observation of relevance to our model since the simultaneous

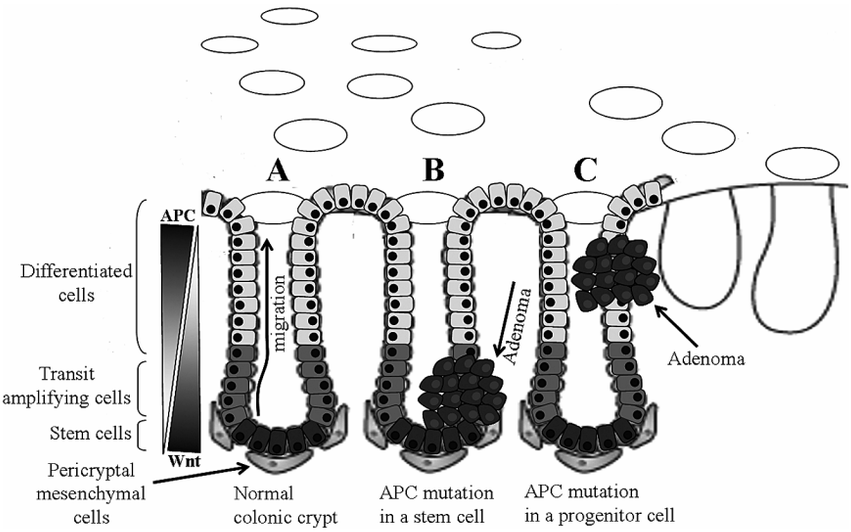

Figure 1. Models of tumorigenesis in the colonic crypt initiated by mutant APC. (**A**) Schematic representation of a normal colonic crypt showing the distinct functional compartments and the unidirectional migratory route of progenitor cells. Stromal mesenchymal cells (arrow) briskly communicate with colonic cells and inform their phenotype. Circles denote the opening of crypts in the colonic lumen. APC is a key intracellular component of a multiprotein complex that restrains Wnt activity. Wnt activity is predominant in the SC zone and proliferative compartment of the colonic crypt; note the inverse expression gradients of APC protein and Wnt pathway along the crypt axis. (**B**) APC mutation occurs in a SC at the base of the colonic crypt. This early genetic hit activates the oncogenic Wnt pathway. Additional genetic changes result in clonal expansion with formation of an adenoma (arrow) which grows bottom-up the crypt axis. (**C**) APC mutation occurs in a migrating progenitor cell after exit from the SC zone. This early genetic hit not only activates the oncogenic Wnt pathway but also perturbs the upward migration of the aberrant clone that is retained in the colonic crypt. Additional genetic changes result in clonal expansion with formation of an adenoma (arrow) which grows top-down the crypt axis. Note that in the present scheme, cancer cell-of-origin and cancer stem cell do not necessarily denote the same cell type.

silencing of both APC alleles is highly improbable, and APC heterozygosity best defines the initial mutational status in a human progenitor clone.

Recently, Song *et al.* (19) using computational modeling and *in vivo* experiments presented evidence that wild-type APC regulates the removal of mutant cells from colonic crypts. The investigators propose that the increasing abundance of APC towards the crypt surface (Figure 1A, this review) maintains a gradient of cellular adhesion that causes hyperproliferative and mutant cells to increase their movement towards the colonic crypt cuff to be ultimately shed into the lumen. Importantly and consistent with our model, mutant APC fails to control cell migration and favors retention of mutant cells in the colonic crypt.

Our hypothesis, supported by mathematical models (20–22), has been further validated by experimental evidence. Thus, van de Wetering *et al.* (23) in an incisive study on the role of catenin-β/TCF4 pathway in the early stages of colonic tumorigenesis have provided convincing evidence that crypt progenitor cells that carry an APC or a β-catenin mutation were able to growth into micro adenomas. Constitutive β-catenin/TCF-4 complex activity imposed a crypt progenitor phenotype on colorectal cancer cells.

Successive studies provided interesting findings related to colonic TA cells as putative cancer cells-of-origin. Barker *et al.* (24) used a *Cre* mouse model to conditionally delete *Apc* colonic $Lgr5^+$ SCs. The simultaneous bi-allelic *Apc* loss resulted in the emergence of a transformed $Lgr5^+$ SC population at the base of the colonic crypts that expanded in time into a macroscopic adenomatous growth. By contrast, the loss of *Apc* in normally short-lived progenitor cells resulted in proliferative foci that rarely advanced to microscopic adenomas, and this observation was interpreted as implying that most of these foci were unable to sustain neoplastic growth. Of note, however, long-term linear tracing analysis has shown that microadenomas in the TA zone of the crypt continuum were present several months after their induction: the mutant clones stubbornly clung to the crypt home and were not lost, as predicted, into the intestinal lumen, leaving open the possibility that additional genetic/epigenetic hits could ultimately pave the way for TA-derived microadenomas to grow into malignant adenocarcinomas.

Notwithstanding the key information that these hallmark studies have provided on colonic crypt SCs, a note of caution, previously mentioned by Medema and Vermeulen (25), is warranted before drawing conclusions pertaining to human colorectal cancer on the basis of findings collected from mouse colonic *Apc* null crypts. Detailed comparison of mutations in both human APC alleles, assessment of constitutive Wnt signaling and severity of the human genetic and sporadic colorectal cancer, have led to the intriguing conclusion that the loss of the remaining wild-type APC allele in a colonic heterozygote APC cell is not a stochastic, random event (26); the inactivation of APC appears therefore not to conform to Kudson's two hits hypothesis related to silencing of tumor suppressor genes in cancer.

This unpredicted divergence has been reported in a number of studies showing that hereditary and somatic APC mutations are selected according to the growth advantage they are able to confer to the tumor cell, to wit, colon cancer phenotypes are directly related to the intra-genomic position of the first APC mutation. Remarkably, the two hits of APC are co-selected to produce an optimal—just right—activation of Wnt signaling (27–29). Consistent with these observations, human mutant truncated APC peptides are mostly stable and retain, like the wild APC type, partial ability to target β-catenin for proteasomal degradation (30), possibly as a safeguard against overexpression of β-catenin liable to harm the cancer cells by upregulating their unwanted apoptotic program. Results using genetically engineered mice carrying different hypomorphic *Apc* alleles have confirmed that Wnt activity at the early stages of colonic tumorigenesis is carefully balanced and dosage-dependent (31), and different phenotypic differences in simultaneous versus stepwise *Apc* loss have been described by Fisher *et al.* (32). These investigators noted that stepwise mutation of murine *Apc* alleles resulted in a faster and a more efficient transformation when compared with simultaneous *Apc* loss. On the basis of these findings, a pertinent question comes to the fore: how representative are *Apc* null mouse models of early colonic tumorigenesis for mechanistic interpretation of the cancer process in the human colonic epithelium?

Additional studies attest to the mounting evidence that colonic progenitor cells can initiate neoplasia outside the stem zone. Davis *et al.* (33) recently described in a mouse model of human hereditary mixed polyposis syndrome a pathogenic mechanism leading to intestinal polyp formation involving the aberrant epithelial expression of morphogens gradients that normally relay instructive signals to target cell populations informing them how to link developmental stages to positional decisions. Gradient disruption led to the formation of ectopic intestinal crypts by Lgr5 negative progenitor cells which accumulated somatic mutations and were capable to initiate intestinal neoplasia, a train of cellular and molecular events likely to occur in the generation of human colonic ectopic serrated polyps.

Notably, a subset of colonic post-mitotic differentiated secretory tuft cells were shown to act as colon cancer-initiating cells; a prerequisite for transformation, however, was the combination of an *Apc* mutation with an inflammatory insult (34).

The capacity of progenitor cells to serve as cancer cells-of-origin has been noted in other regions of the gastrointestinal tract. Thus, Schwitalla *et al.* (35) have recently demonstrated in a genetic model of tumor initiation in mice that epithelial villus differentiated cells are able to activate a dedifferentiation program and revert to SCs bearing the canonical $Lgr\ 5^+$ marker. This was shown to happen in a background of constitutively active β-catenin and concomitant activation of the potent pro-inflammatory NF-kB signaling pathways. Cumulatively, these results are in agreement with our hypothesis and with the 'top down' model of colonic adenoma formation. Moreover, the findings reconfirm the view that constitutive activation of the Wnt pathway alone, while initiating the carcinogenic process, is unable to sustain neoplastic progression. It takes (at least) two to tango, and further genetic changes drive colonic tumorigenesis (12), frequently spurred on by a hostile inflammasome (35,36) or an altered microbiota (37).

Studies using non-gastrointestinal tissues have also questioned the view that normal SCs are invariably the cancer cells-of-origin. Thus, in the setting of breast cancer, non-CSC subpopulations were identified that can readily switch to a CSC state (38). Moreover, human cone post -mitotic differentiated precursors committed to forming light-sensing cells were recently shown to be particularly sensitive to the loss of the retinoblastoma gene 1 (39,40).

Mechanisms underpinning the plasticity of progenitor cells

It is well recognized that by hijacking a key mechanism of embryonic development program, opportunistic cancer cells activate the epithelial-to-mesenchymal transition (EMT) to foster invasion and metastasis (41,42). Intriguingly, EMT plasticity was shown to be closely associated with the acquisition of CSC-like characteristics

by non-CSCs. Thus, non-CSCs of human basal breast cells were shown to be plastic cell populations that can revert to a CSC state by up-regulating the transcription factor Zeb1 (43), a member of Snail superfamily and a key inducer of EMT. Importantly, in the non-CSC population the Zeb1 promoter is in a bivalent 'poised' chromatin configuration and responsive to both chromatin-repressive or chromatin-proficient histone posttranslational modifications. The promoter can promptly switch to an active configuration in response to transforming-growth factor-β, a potent EMT-inducing cytokine. It is worth mentioning here that Zeb1 is a direct target of β-catenin/TCF4 signaling in colorectal cancer (44).

Dedifferentiation is a process whereby differentiated, post-mitotic cells acquire a new phenotype and the capacity to re-enter the cell cycle (45). A wealth of information has shown tha that normal differentiated cells in response to SC ablation or tissue injury may dedifferentiate and revert into stable replicating progenitor cells able to replace lost cells. Thus, radiation- induced loss of $Lgr5^+$ SCs in the intestinal crypts directs Notch ligand $Dll1^+$ differentiated secretory progenitor cells to re-express the canonical SC marker Lgr5 and to replenish the SC pool (46). An additional case in point is the study of Stange *et al.* (47) who, following depletion by 5-fU treatment of gastric SCs in mice, identified a subpopulation of fully differentiated secretory chief cells that became proliferative and were able to regenerate all epithelial lineages, thus acting as SCs. Using continuous multiphoton intravital microscopy and surgical implantation of an abdomenal imaging window in the mouse small intestine, a composite methodological approach allowing the examination of SC dynamics *in vivo*, Ritsma *et al.* (48). after targeted ablation of $Lgr5^+$ SCs, observed the transfer of cells from the TA zone to the SC niche border and clonal expansion of individual TA cells called to the empty SC niche. Apparently, following tissue damage or ablation of SCs, progenitor cells 'fall back' into the vacant SC zone where they establish contact with SC niche signals which instruct the former non-SC cells to acquire SC-like properties. Importantly, there is no need of a pre-existing, quiescent reserve SC pool to be activated in tissues upon damage (49). As aptly stated (50): 'if push comes to shove', reprogrammed progenitors act as *bona fide* adult reserve SCs.

An alternative and attractive view posits that the plasticity of intestinal progenitors might reside, at least partly, on epigenetic signatures shared with SCs. A recent study (51) exploring mouse small intestinal histone marks such as H3K4me2 and H3K27ac associated with chromatin accessibility and transcriptional proficiency, has shown that many intergenic regulatory regions were marked with no evident differences between SCs and their progeny committed to differentiaton. Of note, histone activation was prominent in enhancer regions, an observation consonant with a previous study showing that DNA methylation during differentiation of intestinal SCs was observed predominantly in enhancer sequences (52). These interesting findings signify that regulatory chromatin regions in intestinal cells are open to lineage-specific transcription factors and other microenvinromental agents permitting cell fates to be reverted, with surprising facility, without the emergency signals arising from tissue injury or SC ablation: one may argue that epigenetic plasticity informs cell progenitor plasticity.

The widespread SC heterogeneity and progenitor plasticity in epithelia have been recently reviewed (53,54).

A sustainable model of tumor progression: progenitors to the fore

The inconsistencies that undermine the credibility of the invariable, unidirectional cancer stem model and the emergency of the non-CSC progenitor as a cancer cell-of-origin have been incisively discussed by Chaffer and Weinberg (55), and an alternative model of multistep tumor progression has been proposed by positing that progenitor TA cells after acquiring a heritable change may dedifferentiate and move back into the SC pools. In essence, the model proposes that while CSCs sustain malignant growth and propagate tumorigenesis, they are not necessarily the initial site of the tumorigenic hit. The original sin is borne by progenitor cells outside the SC zone, to wit, cancer cell-of-origin and CSC do not necessarily denote the same cell type.

Conclusion

Cumulative findings indicate that stemness is not an intrinsic prerogative of a pool of privileged SCs but it is lost and reacquired by normal and mutant progenitor cells. Our model as originally proposed (11) did not imply that an APC mutant clone, away from the SC zone and freed from the demandig task of controlling the level and duration of the Wnt pathway, must inevitably re-enter the SC state to foster the tumorigenic process. However, the possibility that mutations first established in colonic progenitor populations are successively reintroduced into a CSC state by an EMT-driven process, akin to the reprogramming process shown in breast tumors (43), is worth considering.

We believe that both the SC and progenitor models pertaining to cancer cell-of-origin are tenable and that a number of potential cancer cells-of-origin exists including SCs,TA and terminally differentiated progenitors. In this scenario, colonic adenoma morphogenesis by the 'bottom-up' or 'top down' routes can occur depending on specific contexts (see also 35). What it is important, however, is that the long-entrenched 'one-way lane' view that SCs represent the *exclusive* cancer cells-of-origin has been robustly challenged by the remarkable plasticity of progenitor cells.

A question of key importance: what is the translational impact of these exciting observations? Obviously, for future therapeutics to succeed, they must take into consideration the finding that non-SC populations are plastic, easily moulded and able acquire, on demand, SC competence. In this scenario, how effective might be a drug targeted exclusively against CSCs? Will killing of CSCs be followed by the swift moving in non-CSCs to occupy the vacant space concomitantly with acquisition of a CSC-like state? Consequently, in addition to therapies aimed to target CSCs, the recurring observation of progenitor cells plasticity in cancer and following tissue injury should inform the design of future anticancer drugs and protocols aimed to tissue-specific regeneration.

Future directions

In the past, the isolation and long-term culture of viable colonic stem and progenitor cells proved to be replete with technical difficulties and results were discouraging. The failure was mainly due to the proneness of colonic epithelial cells to activate the apoptosis program once deprived of crypt habitat (56). Recently, this methodological impasse has been circumvented and single-sorted SCs from human colonic biopsies were able under specific culture conditions to form spheroid-like organoids and to retain multipotency necessary to spawn all cell lineages (57). With this in mind, we propose as an attractive line of investigation to explore whether colonic progenitors once cultured under permissive conditions—for instance, modulating their epigenetic landscape, inducing the EMT program or following exposure to a stem niche-like milieu—are capable to generate SCs, thus implementing in an easily manipulated *in vitro* system the

process of reverse reprogramming, to wit, progenitors to SCs. It will also be of great interest to assess whether reverse programming in colonic differentiated cells is inversely correlated to the degree of their maturity as shown by Tata *et al.* (58) in mouse epithelial fully differentiated airway luminal secretory cells.

The importance of distinct epigenetic signatures at enhancers in cell fate specifications (51,52,59) suggests an additional exciting line of research focused on the identifications of transcription factors involved in the modulation of the epigenetic landcape of progenitor cells during the dedifferentiation act. We suspect that signals flowing from the TGFβ/SMAD pathway play a determining role in this process. More information on epigenetic global and site-specific histone and DNA changes during the acquisition of the dedifferentiated phenotype by colonic progeny cells is urgently needed.

The deliberate pursuing of these rich areas of investigation of relevance not only to colonic crypt cell populations but also to non-intestinal epithelial tissues will undoubtedly add key insights into a cardinal question: how is stemness lost and refound by a progenitor cell?

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