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COMMENTARY

Migrating colonic crypt epithelial cells: primary targets for transformation

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It is widely believed that stem cells are the relevant target cells for colonic cell transformation. Evidence is presented that a proliferative transit daughter cell acquiring a mutant adenomatous polyposis coli gene during upward migration from the crypt base can develop retention abnormalities and permanence in the crypt, thus qualifying as a transformed clone which is retained in the colonic epithelium.

The bottom-up model of colonic tumorigenesis

The steady state of cell growth of normal colonic epithelium is maintained by a dynamic balance between continuing cell renewal and shedding of cells at the surface of the colonic crypts. Stem cell daughters proliferate in the lower part of the crypt, move in a tight cohort toward the upper crypt regions, gradually lose their capacity to divide and finally acquire the differentiated phenotype (1–9). In normal colonic epithelium the sizes of the various functional compartments along the crypt axis are maintained within precise boundaries by multiple homeostatic signals (1–9).

This ordered growth program of the colonic epithelial cells goes into disarray during colonic tumorigenesis. Lipkin (10) earlier proposed a multi-step model for colonic carcinogenesis in which colonic epithelial cells did not repress DNA synthesis during migration along the crypt continuum; they then developed additional abnormal properties enabling them to be retained in the mucosa, and to develop into microadenomas and the various types of adenomatous lesions.

It is widely believed that the relevant target cells during colonic cell transformation are the stem cells (11–16). While the possibility of mutational changes occurring in colonic proliferative daughter cells has been considered, it has been argued (4) that any mutational alteration will be lost in daughter transit cells as they will invariably move out of the crypt soon after birth, unless a genetic change alters concomitantly proliferation, cell-to-cell adhesion interactions and migration, all complex cellular events which are unlikely to be directly genetically linked (4).

In contrast to transit proliferative cells moving steadily towards the crypt surface, slow-dividing stem cells were permanently tethered to a niche near the base of the crypt; therefore, oncogenic mutations may accumulate in these founder, long-lived cells and persist for a time equivalent to pre-cancerous latent periods (from months in rodents to decades in man). Implicit in this view is the tenet that early transformed colonic epithelial cells are the direct progeny of a mutant stem cell: they will produce in time dysplastic crypts that will branch as the lesions expand from the bottom of the crypt.

Top-down models of colonic tumorigenesis

Numerous findings related to the histogenesis of early colorectal adenomas are not consistent with the view presented above. Lightdale *et al*. (17) in an *in vivo* study of proliferation kinetics of human colonic epithelial cells in familial adenomatous polyposis coli, a hereditable form of colorectal cancer, used pulse-delivered tritiated thymidine to observe the following events: (i) a marked shift of the proliferative compartment of colonic epithelial cells from the base of crypts to the crypt surface; (ii) a retrograde migration of adenomatous epithelial cells away from the surface of the mucosa toward the crypt base referred to as ‘intrusion’, as the cells had not yet gained the additional properties required to invade through the basement membrane of the crypt column; and (iii) epithelial cells in the adjacent flat mucosa exibited the normal, upward migration from the crypt base along the crypt continuum.

The retrograde traffic of the transformed cells has also been observed using additional biological markers. Thus, Strater *et al.* (18) and Moss *et al*. (19) reported that, in marked contrast to normal colonic epithelial cells, cells in colonic adenomas expressing apoptosis and TGF-β were located predominantly at the crypt base. Shiff and Rigas (20) have reviewed the evidence for inward growth of colonic adenomatous polyps.

In a recent incisive paper Shih *et al*. (21) have examined the molecular characteristics of cells present in small colorectal adenomas within the crypt columns. They observed that dysplastic cells at the lumenal surface of the crypts exibited genetic changes in the adenomatous polyposis coli (APC) gene associated with functional alteration in β-catenin and an abnormal pattern of cell proliferation, i.e. a retrograde, ‘top-down’ movement of transformed colonic cells towards the lower regions of the colonic crypt. In contrast, cells resident in the base of the same crypts did not show these alterations and were not clonally related to the adjacent cells above them.

A pertinent question posed by the authors was how to reconcile these observations with the stem cell origin of colorectal neoplasia. They advanced two plausible explanations: (i) the first proposed that the stem cell precursors of dysplastic cells reside in the intercryptal zones at the surface of the mucosal epithelium; (ii) the second alternative proposed that dysplastic cells originated in stem cell precursors at the bottom of the colonic crypt migrate passively to the crypt surface; once in the upper region of the colonic epithelium they continue to proliferate and expand into adjacent normal crypt epithelium, pushing the normal tissue downward and gradually replacing it from a top–bottom direction.

In a recent review, Fodde *et al*. (22) suggested that transformation might occur in a fully differentiated cell, which subsequently divides and then invades the normal colonic epithelium from the top-down.

Abbreviation: APC, adenomatous polyposis coli.

We propose below a further alternative possibility that considers genetic changes occurring in migrating daughter cells above the stem cell zone.

Migrating colonic crypt daughter cells as primary targets for transformation with APC mutation the initiating event

The possibilities presented above pertaining to the crypt stem cells as the exclusive targets of colonic carcinogenesis are feasible, if distinct proteins encoded by different genes independently and separately regulate growth and motive signals in normal colonic cells. However, they could be considered less likely if these diverse physiological processes are under the control of a single multifunctional, pleiotropic protein. If so, a germ-line or somatic mutation in a gene coding for the putative protein should result in altering concomitantly both proliferation and migration of a daughter colonic cell.

The APC gene product is a salient example of a multifunctional, pleiotropic protein. The tumor-suppressor APC gene exerts a gatekeeper function (23), directly inhibiting the growth of colonic tumors. In the genetic model of colonic adenoma-to-carcinoma progression characterized by the acquisition of sequential mutations (23,24), those in the APC gene are the earliest alterations required to initiate tumorigenesis, and the expression of wild-type APC gene is lost in 80% of early colonic adenomas (23,24).

The APC polypeptide controls a vast number of key functions related to the ordered growth of intestinal cells including: (i) the steady state levels of β-catenin and its degradation by the ubiquitin–proteasome pathway, thus preventing the formation of the transcriptionally active β-catenin–Tcfs complex which leads to inappropriate activation of several genes, including *c-myc*, cyclin D1, matrilysin and survivin; (ii) maintenance of apoptosis; (iii) cell-cycle progression; and (iv) maintenance of chromosomal stability, thus safeguarding the fidelity of chromosomal segregation (25–31).

Much evidence is available showing that APC binds to the cell cytoskeleton. The protein has been shown to move toward and to cluster near the distal tips of microtubules at the edges of migrating epithelial cells (32–38). While the precise motive signals for the APC movement remain to be elucidated, Jimbo *et al*. (39) have recently reported that APC interacts with proteins of the kinesin superfamily, suggesting that APC is transported along microtubules by these ATP-driven motor molecules.

A functional involvement of APC in cell movement is also sustained by the observation (40) that intestinal cell migration along the crypt axis is altered in histologically normal flat mucosa of Apc *min* mice bearing a mutated Apc allele, suggesting a dominant-negative mode of action for mutant APC. A lack of ordered migration of epithelial cells along the crypt–villus axis was also observed in the earliest adenomas of $Apc\Delta^{716}$ mice (41,42). Hoier *et al*. (43) have shown that inactivation of the APC-related protein APR-1 in *Caenorhabditis elegans* also leads to defect in epithelial cell migration.

Recently, Kawasaki and colleagues (44) reported that the APC gene product is bound through its *armadillo* repeat to the Rac-specific guanine nucleotide exchange factor (Asef) and proposed that APC enhances the activity of Asef, resulting in the activation of Rac, a small G protein, consistent with the view that APC interacts with the actin cytoskeleton (36,45). The functional link between APC and the Rac–Rho signaling pathway is of prime interest as Rho GTPases play key roles in coordinating cellular events involved in cell migration (46).

Fenton *et al*. (47) have recently reported that while EGF and HGF stimulated *in vitro* migration of colonic mouse Apc+/Apc+ cells, murine cells heterozygous for Apc were less migratory in response to challenge with the growth factors.

As APC is associated also with the plasma membrane (38), it is likely that APC mutations, leading to changes in the free pool of β-catenin, alter the relative adhesiveness of epithelial cells and the integrity of the cadherin–catenin adhesion complexes (48). Thus, in murine epithelium, cell migration along the crypt–villus axis was slowed by the forced expression of a N-terminal truncated β-catenin (49): cells did not comply with the normal upward, vectorial migration and mixed with neighboring crypts. Carothers *et al*. (50) have convincingly shown that the tumor-promoting effect of the Apc mutation in the intestine of *min* mice involves a defect in cadherin-mediated adhesion. Hamada and Bienz (51) have recently observed in *Drosophila* that an APC protein homolog is involved in the maintenance of cellular junctional complexes.

Cumulatively, the above findings indicate that, as pointed out previously by Potter (52), a mutation in APC creates simultaneously all the abnormalities in adhesion, migration—and proliferation—for an affected cell to grow into a polyp.

A logical corollary of these observations affords a further alternative explanation for the origin of crypt target cells in colorectal carcinogenesis: namely that a proliferative transit daughter cell derived from a normal crypt progenitor cell, and acquiring a mutant APC molecular signature during upward migration from the crypt base, not only will exhibit aberrant mitotic behavior but with concomitant retention abnormalities could acquire a stem cell phenotypic characteristic, i.e. permanence in the crypt, thus qualifying as a transformed clone resident in the colonic epithelium. This view does not preclude the possibility that an APC mutation may occur in a stem cell: the aberrant cell progeny will be also retained in the colonic crypt.

In the morphogenesis of colonic polyps (21) genetic alterations of APC were found in small sporadic colonic adenomas (average diameter 2 mm) collected on the surface of colonic crypt. At this stage of colonic dysplastic growth ras mutations are rare (22,23,53) and dysplastic adenomas measuring <2 mm have been shown to exhibit increased expression of COX-2, possibly as a result of APC inactivation (54–56).

The earliest cellular event leading to colonic adenoma formation has been described as a failure of epithelial cells to repress DNA synthesis as they migrate toward the crypt surface (10,57). We know now that when abnormally proliferating colonic proliferating cells reach and are retained at the luminal surface, they can form microadenomas exhibiting APC mutations (21). Intramucosal focal areas of dyspasia have been described further during the evolution of colonic neoplasia (58), and somatic APC mutations have been found in a small subset of human aberrant crypt foci (59). The APC mutations were associated with manifestation of tissue dysplasia and therefore have been considered key molecular determinants for progress to more advanced neoplastic lesions in the colonic epithelium (22,60,61).

The expression of the APC protein in the normal colonic crypt shows a typical topological distribution, i.e. very feeble or absent in the crypt base but increases sharply toward the upper third of the crypt and the luminal surface (62–64). Shih

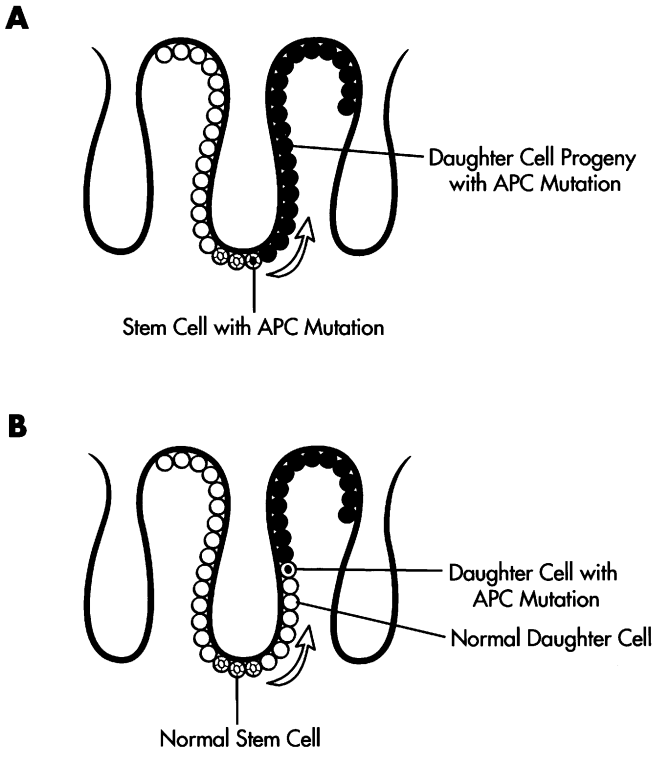

Fig. 1. A colonic cell with an APC mutation leading to enhanced proliferation and chromosomal instability acquires a transformed phenotype. The aberrant clone becomes permanently retained in the colonic crypt due to migration abnormalities resulting from loss of wild-type APC protein. (**A**) APC mutation occurs in a stem cell and cell progeny retains the abnormal APC molecular signature. (**B**) APC mutation occurs in a migrating daughter cell above the stem cell zone. Note that cells with APC mutation are found in the upper crypt and its lumenal surface, whereas stem cells at the base of the crypt are normal. In both models of colonic tumorigenesis, clonal expansion of cells with APC mutation leads to the formation of a microadenoma.

et al. (21) have detected the expression of APC transcripts at the base of small adenomatous colonic crypts. It is noteworthy that cells at that position were shown to be morphologically normal and to express the wild-type APC DNA sequence.

The possibility proposed above suggesting that a permanently fixed genetic alteration in colonic cells originates in migrating daughter cells above the stem cell zone, is consistent with morphological findings recently reported, i.e. that dysplastic cells with an APC mutation are found at the lumenal surface and in the upper zone of the colon crypt, whereas the cells at the bottom of the crypt appear to be morphologically normal (21).

In conclusion, we surmise that an APC mutation(s) in a migrating and proliferative colonic epithelial cell is sufficient to transform the daughter cell into an incipient aberrant clone retained in the colonic mucosa (Figure 1) gradually growing into a microadenoma. Whether silencing of one APC allele is sufficient to initiate this process, possibly by haploinsufficiency (27,65–67) or by a dominant-negative effect (27,40,51,68), or whether loss of both APC gene copies is required (23), remains an open and challenging question; each possibility would differently affect the frequency of acquisition of the abnormal phenotype by a colonic daughter transit cell. The resulting minute polyp might expand in time and intrude into the intercryptal space showing the characteristic feature of top-down morphogenesis. Once the small adenoma is formed, additional gene mutations (e.g. K-ras, p53) and epigenetic changes (23,24) would be mandatory for an increase in size and the acquisition of a more aggressive phenotype, a process taking months in rodents and decades in humans

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