Corruption of homeostatic mechanisms in the guanylyl cyclase C signaling pathway underlying colorectal tumorigenesis

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Key words: colon cancer, homeostasis, proliferation, chromosomal instability, migration and adhesion, apoptosis, guanylyl cyclase C, CDX2, APC, p53

Abbreviations: AOM, azoxymethane; APC, adenomatous polyposis coli; BrdU, 5'-bromo-2'-deoxyuridine; GCC, guanylyl cyclase C; BAX, BCL2-associated X protein; BAK, BCL-2 homologous antagonist/killer protein; CDX, members of caudal-rostral homeobox gene family; DCC, deleted in colorectal cancer; Dkk-1, dickkopf 1; FAP, familial adenomatous polyposis; K-RAS, V-Ki-ras2 kirsten rat sarcoma viral oncogene homolog; LOH, loss of heterozygosity; MSH, melanocyte stimulating hormone; mTOR, mammalian target of rapamycin; p53, tumor protein 53; PUMA, p53 upregulated modulator of apoptosis; ROS, radical oxygen species; SMAD, mothers against decapentaplegic homolog family; TGFβ, transforming growth factor β; TCF, T-cell factor; TUNEL, terminal deoxynucleotidyl transferase-mediated dNTP-biotin nick end labeling; ST, bacterial heat-stable enterotoxin

Colon cancer, the second leading cause of cancer-related mortality worldwide, originates from the malignant transformation of intestinal epithelial cells. The intestinal epithelium undergoes a highly organized process of rapid regeneration along the crypt-villus axis, characterized by proliferation, migration, differentiation and apoptosis, whose coordination is essential to maintaining the mucosal barrier. Disruption of these homeostatic processes predisposes cells to mutations in tumor suppressors or oncogenes, whose dysfunction provides transformed cells an evolutionary growth advantage. While sequences of genetic mutations at different stages along the neoplastic continuum have been established, little is known of the events initiating tumorigenesis prior to adenomatous polyposis coli (APC) mutations. Here, we examine a role for the corruption of homeostasis induced by silencing novel tumor suppressors, including the intestine-specific transcription factor CDX2 and its gene target guanylyl cyclase C (GCC), as early events predisposing cells to mutations in APC and other sequential genes that initiate colorectal cancer. CDX2 and GCC maintain homeostatic regeneration in the intestine by restricting cell proliferation, promoting cell maturation and adhesion, regulating cell migration and defending the intestinal barrier and genomic integrity. Elimination of CDX2 or GCC promotes intestinal tumor initiation and growth in aged mice, mice carrying APC mutations or mice exposed to carcinoogens. The roles of CDX2 and GCC in suppressing intestinal tumorigenesis, universal disruption in their signaling through silencing of hormones driving GCC, and the uniform overexpression of GCC by tumors underscore the potential value of oral replacement with GCC ligands as targeted prevention and therapy for colorectal cancer.

Introduction

In the oncogenic model of colorectal cancer, epithelial cells progress through a series of morphological stages driven by underlying genetic mutations that result in the transformation into an invasive carcinoma. Damage to the genome exceeds the capacity for repair, producing irreversible mutational changes that result in the neoplastic phenotype. Genetic and epigenetic changes, reflecting corruption of DNA damage sensing and repair circuits, in conjunction with the uncoupling of apoptosis, lead to the gain-of-function of oncogenes and the concomitant loss-of-function of tumor suppressors.1 In turn, these changes disrupt intestinal homeostasis producing hyperproliferation, amplification of genetic instability, altered migration, disrupted adhesion, resistance to apoptosis and failure to repair damaged cells. Moreover, in colorectal cancer, alterations driving transformation often occur in a specific sequence, suggesting that different genes, and the homeostatic processes they regulate, play essential roles at different stages along the transformation continuum.

Genetic alterations important to the colorectal tumorigenesis sequence have been identified, including APC, β-catenin, Axin, MSH, K-RAS, SMAD and p53, among others. Adenomatous polyposis coli (APC) is mutated in more than 80% of sporadic colorectal tumors and germline mutations in APC underlie the inherited intestinal neoplastic syndrome Familial Adenomatous Polyposis. APC, as a “gatekeeper” for colon cancer, inhibits cell proliferation,2 regulates cell migration3 and maintains chromosomal stability4,5 to defend intestinal homeostasis.6 Mutations in APC or its downstream effector β-catenin, initiate the growth of small benign polyps. However, these mutations are not sufficient to support the progression of hyperplastic lesions to invasive carcinoma. Other signaling pathways, including transforming growth factor β (TGFβ) family members and TP53 (p53), are required...
for tumor progression. The TGFβ family, a group of small peptide hormones, negatively controls colon cell growth through SMAD4, and their silencing through mutations of canonical TGFβ receptors contributes to neoplastic progression. A well-established genomic guardian, maintains genomic integrity by inhibiting cell growth through cell cycle arrest, inducing apoptosis, and promoting DNA repair in response to DNA damage.

An emerging paradigm suggests that early events contributing to the initiation of the tumorigenic continuum that precede mutations in APC and its downstream effectors convey an evolution-ary advantage to intestinal epithelial cells, which is essential to transformation. Mutations of CDX2, a tissue-specific homeodomain transcription factor regulating the development of the intestine, and silencing of guanylyl cyclase C (GCC) signaling, the intestinal receptor for the paracrine hormones guanylin and uroguanylin, and a target gene of CDX2, characterize the earliest identifiable stages along the transformation continuum. Mutations in CDX2 and dysregulation of GCC signaling, which reflect silencing of guanylin and uroguanylin expression, could contribute to the loss of genomic integrity and the development of mutations in APC and its downstream effectors, reflecting loss of normal proliferative and DNA quality control, and predisposing epithelial cells to tumor initiation.

The Intestinal Crypt-Villus Axis

The intestinal mucosa is covered by a single layer of epithelial cells, which is organized in vertical anatomical units underlying specialized organ functions. In the small intestine, the major organ for nutrient absorption, villi projecting into the lumen and flat-topped crypts embedded in the mesenchyme expand the secretory and absorptive surface area and provide the structure supporting digestive processes. In contrast, the large intestine exhibits a comparatively smooth surface with tubular crypts embedded in the colonic mesenchyme. Intestinal epithelial cells cover the luminal surface of the crypt-villus axis and provide a physical barrier between systemic and mucosal compartments by sealing epithelial cells with tight junctions. A highly dynamic process of continuous epithelial cell proliferation, migration, terminal differentiation, apoptosis and shedding maintains the structural and functional integrity of the crypt-villus axis. Thus, tubular crypts embedded in the mesenchyme form the proliferating zone in the crypts of the small intestine and colon, and regenerative stem cells at the bottom of the crypts give rise to rapidly proliferating daughter cells. In contrast to crypts, villi are covered by permanently differentiated cells projecting into the lumen, supporting their digestive and absorptive functions.

Stem cells proliferate relatively slowly and their regeneration rate is not fast enough to support intestinal epithelial renewal. Thus, rapid transit cell proliferation will amplify the supply of cells to meet the demand of epithelial renewal. Transit cells, which initiate a program of terminal phenotypic maturation triggered by as yet undefined signals, lack the ability to proliferate indefinitely while they are migrating along the crypt-villus axis. Transit cells give rise to four principal cell types characterizing the crypt-villus axis: absorptive enterocytes, goblet cells, endocrine cells and Paneth cells. Enterocytes, which constitute approximately 80% of epithelial cells, are polarized columnar cells mediating digestive functions, such as hydrolysis and absorption of nutrients, and secretion of fluid and electrolytes. The main characteristics of mature enterocytes include well-developed microvillus brush border membranes containing key functional proteins mediating cognate digestive and absorptive functions. Goblet cells are mucin-secreting cells that protect the intestinal lumen and facilitate nutrient absorption by enterocytes. Enterendocrine cells, which constitute 1% of epithelial cells, produce autacoids, peptides and hormones and are part of the neuroendocrine system in the intestine, with paracrine and autocrine functions locally and endocrine functions supporting systemic activities, for example in the hypothalamus. Finally, Paneth cells protect the mucosa by secreting antimicrobial peptides, digestive enzymes and growth factors into the lumen. Paneth cells, absent in the colon, are one of the proposed mechanisms defending the small intestine against tumorigenesis by mediating innate immune responses to intestinal pathogens.

Enterocytes, goblet and enteroendocrine cells migrate toward the villus tip (small intestine) or surface of the crypt (colon) where they initiate a program of apoptosis or are exfoliated into the intestinal lumen by as yet unknown mechanisms. In contrast, Paneth cells differentiate during a 5–8 day downward migration to the crypt base. Turnover of enteroendocrine and Paneth cells is relatively slow compared to other cell types in the intestine. Rapid cell renewal and the transition from proliferation to differentiation require tight homeostatic control of subordinate cell physiological circuits, including proliferation, differentiation, migration and apoptosis in epithelial cells. Disruption of these circuits corrupts normal structure and function contributing to intestinal tumorigenesis.

CDX2 and GCC Signaling Regulate Intestinal Homeostasis

CDX2, a member of the homeodomain transcription factor CDX family (CDX1 and CDX2), is expressed in the intestinal epithelium during embryogenesis and in adults. In contrast to CDX1, which localizes to the progenitor cell compartment regulating cell proliferation that supports intestinal renewal, CDX2 is expressed throughout the crypt-villus axis and maintains intestinal homeostasis by regulating the transition of cells from proliferation to differentiation. CDX2 regulates the expression of intestinal lineage genes in specific regions of the intestine, such as sucrase-isomaltase in the small intestine, carbonic anhydrase I in the colon and GCC in the small intestine and colon. CDX2 is also expressed in intestinal metaplasia of stomach and esophagus, promoting the transition to the intestinal epithelial cell phenotype, reflecting an anterior homeotic shift. CDX2 opposes intestinal tumorigenesis by maintaining homeostasis, which is required for intestinal epithelial renewal. Indeed, mice harboring mutations of CDX2 develop spontaneous colon polyps. Moreover, CDX2 elimination potentiates tumor initiation and growth in the colon of Apc mice through hyperproliferation,
with dysregulated G1/S transition and increased chromosomal instability.35

GCC, a downstream transcriptional target of CDX2, maintains intestinal homeostasis by restricting proliferation and promoting differentiation.19,36 Targeted elimination of GCC signaling in mice (Gucy2c-/-) increases crypt length along a decreasing rostral-caudal gradient by disrupting component homeostatic processes.36 Crypt expansion reflects hyperplasia of the proliferating compartment, with an increase in rapidly cycling progenitor cells and reciprocal reduction in differentiated cells, including Paneth and goblet, but not enteroendocrine cells.36 Moreover, crypt hyperplasia in Gucy2c-/- mice is associated with adaptive increases in cell migration and apoptosis. Further inactivation of GCC signaling promotes intestinal tumor initiation and growth in ApcMin/+ mice heterozygous for the Apc allele, and in mice exposed to the carcinogen, azoxymethane (AOM). In the context of uniform disruption of GCC signaling during human colorectal carcinogenesis, reflecting the silencing of guanylin and uroguanylin, the endogenous paracrine hormones for GCC, these studies suggest GCC signaling also suppresses intestinal tumorigenesis by coordinating homeostatic circuits required for intestinal epithelial renewal.19,20

These previously under-appreciated roles of CDX2 and GCC signaling in maintaining intestinal homeostasis and the near-universal mutation of CDX2 and/or silencing of GCC signaling early along the transformation continuum suggest that dysregulation of these signaling pathways contributes to disruption of intestinal homeostasis, reflecting hyperproliferation and loss of genomic integrity, predisposing epithelial cells to intestinal tumor initiation.19,20

Cell Proliferation and Intestinal Tumorigenesis

Intestinal epithelial renewal requires the availability of a continuous supply of cells produced by proliferation. In crypts, cell proliferation is predominantly regulated by the Wingless signaling cascade, which provides a unique microenvironmental niche for maintaining and activating proliferating cell reservoirs. Upon Wingless/Wnt signal activation, ß-catenin in the cytoplasm translocates to the nucleus and binds to Tcf transcription factors to generate a complex that activates downstream target genes. Abrogation of Wnt signaling by removal of Tcf4 or ß-catenin or by overexpression of the Wnt inhibitor, Dickkopf 1 (Dkk-1), results in a complete loss of proliferation and death of the mouse five days after birth.37 On the other hand, intestinal epithelial cell renewal is highly controlled and restricted by multiple anti-proliferative mechanisms. Disruption of these circuits produces continuous cycling of DNA replication and cell division. In turn, these effects result in cell hyperplasia and accumulation of mutations that potentiate hyperproliferation, prevent terminal differentiation and prevent apoptosis, which ultimately establishes the invasive carcinoma phenotype.

APC is a negative regulator of the Wingless signaling cascade. Intestine-specific inactivation of APC in mice disrupts Wnt signaling, producing nuclear accumulation of ß-catenin and mortality five days after birth. APC-deficient cells in the intestine maintain a “crypt progenitor-like” phenotype. Moreover, beyond a greater number of proliferating cells in the crypt of APC-deficient mice, their spatial organization is altered and cells in S phase are distributed throughout the elongated crypts rather than restricted to the lower two thirds. Furthermore, altered proliferation is associated with accumulation of dephosphorylated ß-catenin, which is resistant to degradation, in APC-deficient mice.4 In turn, ß-catenin activates Wnt-downstream target genes, including cyclinD1, and promotes intestinal cell growth.2,35 Disruption of proliferative homeostasis, mutually reinforced by Wnt signaling and APC mutation, leads to overgrowth of un-differentiated cells contributing to intestinal tumorigenesis. Inactivation of APC is also recognized as a key early event in the development of human sporadic and inherited colorectal cancers. Patients with germline mutations of APC develop numerous colorectal polyps,39 and targeted mutation of Apc in mice results in multiple intestinal tumors.40

Interestingly, targeted silencing of CDX2 and GCC signaling promotes tumor initiation in the colon ApcMin/+ mice,20,35 which suggests that mutations of these genes prior to APC create an evolutionary advantage in hyperproliferation for intestinal epithelial cell transformation. Indeed, CDX2, a key transcription factor mediating intestinal development, is frequently mutated in human colorectal cancer.14 Similarly, expression of the endogenous hormones for GCC, guanylin and uroguanylin, is uniformly lost at the early stages in human and mouse intestinal tumorigenesis.18,41 In that context, elimination of CDX2, GCC and guanylin increases the size of the proliferating crypt compartment, the number of proliferating cells in that compartment, and accelerates their cell cycle.20,35,36,42 These effects are potentiated by genotoxic insults, revealed as hyperplasia of normal intestinal epithelium in Gucy2c-/- mice carrying Apc mutations (ApcMin/+), or exposed to AOM. Moreover, corruption of the proliferative restriction and acceleration of the cell cycle by eliminating CDX2 or GCC signaling promote tumor initiation and growth in ApcMin/+ and AOM-treated mice, reflected by an increase in the number and size of adenoma, and the associated crypt hyperplasia in normal adjacent mucosa.20

Patients with germline mutations of APC do not necessarily develop colorectal cancer, although they are at much higher risk for intestinal neoplasia than the general population. Additional genetic alterations are required for tumors to form in the colon. TGFß and SMAD are members of a signaling pathway frequently mutated subsequent to APC in the colorectal carcinogenesis sequence. Targeted silencing of TGFß receptor43 and SMAD244 and SMAD447 promotes tumor progression and invasion in ApcMin/+ mice by accelerating cell proliferation. Interestingly, inactivation of TGFß receptors increases both colonic tumor number and size while inactivation of Smad2 in ApcMin/+ mice does not change the total number of tumors, but increases mortality reflecting intestinal obstruction caused by large tumors. These observations suggest that TGFß/SMAD signaling is implicated in colorectal tumor progression and invasion by predominantly restricting cell proliferation.43,44

Taken together, disruption of intestinal proliferative homeostasis reflecting mutations in APC and alterations in genes...
occurring before and after APC mutations, corrupts the organization of the crypt-villus axis. Disruption of homeostatic integrity results in a niche susceptible to genotoxic insults and overgrowth of progenitor cells, producing neoplastic transformation and hyperproliferation required for intestinal tumor growth.

**Chromosomal Instability and Intestinal Tumorigenesis**

Colorectal cancer arises through a series of morphologic changes, corresponding to specific gene mutations at each stage, which provide a selective survival advantage continuously expanding the pool of transformed cells. In turn, sequential genotoxic insults to these hyperplastic cells induce multiple mutations in greater numbers of genes producing tumor progression, invasion and metastasis. In humans, FAP patients require about 20 years to lose their functional APC allele. Similarly, in sporadic colorectal cancer, progression from adenoma to metastatic carcinoma requires 20–40 years. In both cases, genetic instability plays a central role in initial transformation of progenitor cells and accelerating the rate of mutation in transformed cells mediating tumor progression.

Chromosomal instability and aneuploidy are classic characteristics of cancer and predictive of poor prognosis. Chromosomal instability reflects DNA damage that exceeds the capacity for DNA damage repair, associated with a failure to eliminate damaged cells. DNA damage in intestinal epithelial cells is caused by endogenous and exogenous genotoxic insults. The predominant endogenous insults are from reactive oxygen species (ROS) as side products of oxidative metabolism that supports rapid proliferation of crypt cells. Exogenous damage reflects a variety of environmental mutagens, for example, alkylating agents. DNA damage repair, including damage detection, assessment and mutational repair through recruitment of repair machinery, is facilitated by suspending cell cycle progression and promoting apoptosis through cell cycle checkpoint-dependent mechanisms. Moreover, chromosomal instability and proliferation are mutually-reinforcing. Beyond the expanded potential for linearly propagating somatic mutations in rapid proliferating cells, cells in S phase with unwound and accessible double-strand DNA are more susceptible to genotoxic insults. Further increased progression through G1 and premature entry into S is associated with amplification of genetic instability.

APC maintains chromosomal fidelity through mitotic checkpoint mechanisms, and this effect is independent of proliferative restriction reflecting antagonism of β-catenin. Further, chromosomal instability producing aneuploidy, revealed by mutations of APC, occurs at the earliest stages of colorectal cancer progression, preceding deregulation of β-catenin. During mitosis, APC regulates spindle assembly, orientation and chromosomal segregation in human colon cancer cells or embryonic stem cells from ApcMin/+ mice. More specifically, APC localizes to the ends of microtubules embedded in kinetochores and forms a complex with the checkpoint proteins Bub1 and Bub3. Interaction of APC with microtubules of the mitotic spindle is mediated by the adaptor, EB1, which localizes at the midplane of the mitotic body and is required for proper spindle assembly in Drosophila and positioning in yeast. Moreover, APC mutations in vivo lead to cytokinetic failure, mitotic defects and tetraploidy in intestine, associated with disoriented spindles, misaligned chromosomes and tetraploid progenitor cells in the crypts. These defects are observed in morphologically normal crypt cells in ApcMin/+ mice with normal levels of β-catenin expression and sub-cellular distribution.

Targeted inactivation of CDX2 or GCC increases chromosomal instability in normal intestinal epithelial cells prior to mutation of the second allele of APC in the colon of APC deficient mice. Deletion of one allele of Cdx2 in mice results in spontaneous colon cancer and dramatically (6 folds) potentiates tumor multiplicity in colons of APC mutant mice through mTOR-mediated chromosomal instability. Indeed, increased tumor initiation and growth by Cdx2 deletion is associated with hyperproliferation quantified by Ki67 staining together with other proliferative markers and genetic instability quantified by anaphase bridge index in normal intestinal crypts. These changes in the pre-transformed stage create a selective survival advantage for transformed cells, amplifying tumor initiation and growth reflected by a higher frequency of loss of heterozygosity (LOH) of Apc in tumors. Interestingly, a deficiency of GCC in the intestine compromises genomic integrity as quantified by increased DNA oxidation and double-strand DNA breaks in crypt cells. Indeed, GCC signaling reduces the production of DNA damage by modulating the reprogramming of metabolism from glycolysis to oxidative phosphorylation and characterizing the switch from proliferation to differentiation. In turn, metabolic reprogramming suppresses ROS production in crypt cells or promotes DNA damage repair, defending genomic integrity. The combination of reduced DNA damage and enhanced DNA damage repair contributes to maintenance of genomic integrity in GCC-expressing mice, although the precise contribution of GCC signaling to steady-state maintenance of the genome, including damage detection and assessment, mutational repair, and the associated coordination of replicative decision making, remains to be defined. These findings suggest that CDX2 mutations and silencing of GCC signaling may provide an environment in the earliest stages of neoplasia in which chromosomal instability together with hyperproliferation, as a self-reinforcing mechanism, lead to further genetic damage and tumor promotion and progression.

Mutations in p53, a well-established guardian of genomic integrity, also are frequently observed in colorectal cancers, although at relatively late stages along the transformational continuum. In this context, it is interesting to note that p53 deletion alone is not sufficient to initiate intestinal tumorigenesis. Chromosomal integrity is maintained by p53 through regulation of gene transcription in response to DNA damage. Upon genotoxic insult, p53 senses the DNA damage and activates the transcription of p21 to arrest the cell cycle and prevent replication of damaged DNA. Also, p53 activates the transcription of the mediators of DNA damage repair and apoptosis to selectively remove damaged cells beyond repair. Constitutive and
inducible elimination of p53 in intestinal epithelial cells does not alter crypt-villus homeostasis under physiological conditions, although epithelial cells in p53-null mice fail to undergo apoptosis following exposure to radiation. Moreover, loss of p53 does not amplify the morphological changes in the crypt-villus architecture following Apc loss. Eliminating p53 minimally impacts tumor multiplicity, but dramatically enhances the invasiveness of intestinal adenomas in Apcmin/+ mice. These data suggest that p53, regulating chromosomal fidelity and apoptosis, but not proliferation, in normal intestinal epithelium, plays important roles in tumor progression and malignant transformation in the late stages of colorectal carcinogenesis rather than in early lesion development. In contrast to the roles of p53 in colon cancer pathophysiology, targeted p53 activation by a p53 modulator, CP-31398, dramatically reduces tumor number in Apcmin/+ mice, thereby suppressing proliferation while promoting apoptosis in the colon.

In summary, regardless of the precise sequence of genetic events contributing to colorectal carcinogenesis, genetic alterations contributing to hyperproliferation and loss of genomic quality control will lead to further damage through a self-reinforcing cycle. Chromosomal instability, as a hallmark of colon cancer can promote initiation and progression of colorectal carcinogenesis by increasing the rate of gene alterations, creating the niche required for tumor evolution.

Cell Death (Apoptosis) and Intestinal Tumorigenesis

Spontaneous cell death occurs predominantly in the lower pole of the crypts, where the progenitor cells are located, and at the villus tips, where cells are shed into the intestinal lumen. Cell death in the lower crypts exhibits the classical characteristics of apoptosis, including apoptotic bodies and condensed nuclei, which deletes excess or DNA-damaged cells. On the other hand, mechanisms mediating cell death at villus tips remain undefined, because these cells rarely exhibit apoptotic morphology and their nuclei are enlarged rather than condensed. Some studies support the apoptotic hypothesis, reflecting the identification of DNA fragmentation and expression of BAX and cleaved caspase 3 at villus tips. Regardless of the detailed mechanisms, cell death along the crypt to villus axis is an important event controlling and stabilizing the overall cell population by deleting excess cells and defending genetic integrity by removing DNA-damaged cells. In that context, disruption of mechanisms controlling cell death may contribute to tumorigenesis in intestine.

The role of APC in apoptosis was revealed in human colon cancer cells by overexpression of APC. Expression of APC in APC-inactive human colon cancer cells leads to diminution of cell growth by inducing cell death through apoptosis. However, the role of APC in apoptosis has been challenged in animal studies in both Apcmin/+ mice and inducible APC knockout mice. Apcmin/+ mice exhibit a decrease of apoptosis in enterocytes quantified by TUNEL, which appears to support previous observations. However, this effect on apoptosis is associated with a concomitant 45% decrease in proliferation measured by PCNA staining compared to wild-type littermates. Therefore, decreased apoptosis in Apcmin/+ mice might reflect reduced proliferation in intestine. Further, inducible inactivation of APC increases apoptosis, quantified by caspase 3 staining with a concurrent increase in cell proliferation. Interestingly, apoptotic cells in APC-deficient mice are enlarged, reflecting cell death at the G1/M checkpoint resulting from mitotic catastrophe, consistent with the hypothesis that APC regulates chromosomal stability through mitotic checkpoint mechanisms. Alterations in cell death that are proportional to changes in proliferation in the intestine suggest that apoptosis compenses for uncontrolled proliferation associated with chromosomal instability induced by APC mutations by removing excess or damaged cells. In that context, APC might not play a direct role in mediating cell death.

Surprisingly, selective inactivation of apoptosis in intestinal epithelial cells does not induce spontaneous tumorigenesis, even though mice with targeted inactivation of BAX and BAK or p53 exhibit compromised spontaneous or induced apoptotic responses in intestine. As mentioned above, cell death contributes to intestinal homeostasis by removing excess or damaged cells. Mice with compromised apoptosis exhibit colonic hyperplasia with altered differentiation, and are more susceptible to carcinogen-induced formation of aberrant crypt foci. Overexpression of CDX2 in human colon cancer cells increases apoptotic sensitivity, suppressing tumor growth in mice. Furthermore, elimination of CDx2 in mice amplifies susceptibility to intestinal tumorigenesis following AOM challenge, which is associated with a 50% reduction of apoptosis in colonic cells. In contrast, a role for GCC in regulating apoptosis in intestine remains undefined. As mentioned above, the impact of CDX2 and GCC signaling on apoptosis as a mechanism contributing to intestinal tumorigenesis is likely in the context of their effects on restricting proliferation and maintaining genetic integrity. Similarly, other key gate keeper genes important for colon cancer progression, including Netrin, the ligand for DCC; PUMA and p21, major downstream target products of p53; inhibit intestinal tumorigenesis exclusively by sensitizing apoptotic responses in the context of compromised proliferative controls.

Taken together, apoptosis is one mechanism, beyond cell migration, that removes excess and damaged cells from the crypt-villus axis and, together with mechanisms controlling proliferation, maintains the homeostatic balance between proliferating and differentiated cell populations. The contribution of apoptosis to dysregulation of intestinal homeostasis in tumorigenesis, especially in cancer initiation, appears to reflect mechanisms controlling proliferation and genetic integrity, rather than a primary mechanism underlying transformation. In that respect, the intestinal epithelium is one of the most rapidly renewing tissues in adults, requiring a robust cell supply provided by proliferation to support regeneration and corruption of that proliferative mechanism is central to the initiation of intestinal tumorigenesis. Certainly, failure of programmed cell death to remove damaged cells beyond repair can be a means of propagating chromosomal instability and cell transformation in the context of rapid proliferation driving homeostatic regeneration.
Cell Migration, Adhesion and Intestinal Tumorigenesis

Cell migration from the bottom of crypts to villus tips is tightly controlled to complete the regenerative process without compromising the integrity of the epithelial barrier, although the underlying mechanisms are not completely understood. Migration of epithelial cells is an active process, rather than passive movement in response to cell replacement from crypts by rapid proliferation. APC is a dominant regulator of enterocyte migration, and dysregulation of this process may contribute to intestinal tumorigenesis. In mice, intestinal epithelial cells renew every 48–72 hours as quantified by tracking labeled progenitor cell migration with 3H-thymidine or BrdU. Enterocyte migration along the crypt-villus axis is decreased by 25% in Apc-/- mice, and those cells exhibit increased residence time and reduced adhesion junction structural integrity reflected by a decrease in membrane-bound E-cadherin and the dissociation of E-cadherin from β-catenin. Moreover, enterocyte migration is completely abrogated in Apc-/- mice and crypts are elongated and populated by rapidly proliferating, but stationary, epithelial cells. Instead of moving toward villus tips, Apc-/- cells accumulate in upper regions of crypts, forming abnormal crypt foci in the absence of β-catenin nuclear accumulation. Following this initial corruption of cell migration, hyperproliferating cells in abnormal crypts progress to micro- and macro-adenomas.

Migration, cell adhesion and proliferation exhibit reciprocal regulation that maintains epithelial homeostasis. Disruption of any individual component process breaches intestinal barriers, including physical and molecular barriers, which causes systemic genotoxicity promoting local and systemic tumorigenesis. Both delay and acceleration of cell migration disrupts intestinal integrity to promote tumorigenesis. Conversely, CDX2 and GCC signaling defend intestinal barrier integrity by coordinating cell proliferation and migration, and regulating cell adhesion. Mutating CDX2 or eliminating GCC signaling early in transformation induces a tumorigenic microenvironment by disrupting the intestinal barrier, inducing inflammation and APC dysfunction which, in turn, produce the cell accumulation that characterizes tumor initiation.

In summary, cell migration, another mechanism beyond cell death and shedding, removes cells from crypts and, together with mechanisms controlling proliferation, maintains the homeostatic balance between proliferating and differentiated cell populations. Further, cell migration, in coordination with adhesion and proliferation, maintains barrier integrity, defending against systemic exposure to genotoxic and pathogenic insults from the gut lumen. Indeed, disruption of pathways regulating cell migration and adhesion breaches the intestinal barrier, producing local and systemic chromosomal instability predisposing to mutations of APC and other genes contributing to colorectal tumorigenesis. In turn, APC mutations amplify migratory inhibition in crypts and, together with hyperproliferation inherent in that niche, promote genomic instability leading to tumor initiation.

Novel Strategies of Targeted Colon Cancer Prevention and Therapy

There is an unmet clinical need to bridge the gap between existing screening paradigms and barriers promoting underutilization of primary chemoprevention strategies by the at-risk population including aged adults and patients carrying inherited germline mutations. In that context, elucidating the detailed mechanisms underlying intestinal homeostasis and colorectal tumorigenesis identifies targets for chemoprevention. The novel roles of CDX2 and GCC in homeostatic mechanisms: restricting cell proliferation, maintaining genomic stability, regulating cell migration and defending intestinal integrity, and in suppressing tumor initiation and progression, especially in the context of APC mutations, underscore the utility of CDX2 and GCC as targets for colon cancer prevention. Ideal targets include oncogenes, which exhibit overexpression or over-activation in tumors compared to normal tissues, or tumor suppressors, which are mutant or silenced in tumors, although reconstitution of altered tumor suppressor signaling is technically more challenging than suppressing over-active oncogenes. On the other hand, ideal chemopreventive agents exhibit activities at the target in a therapeutically achievable range, with limited or absent collateral activities in non-target tissues, for optimum safety.

GCC may fit the criteria of the ideal target for colorectal cancer prevention and represent a unique model for reconstitution of signaling underlying tumor suppression. GCC expression is primarily restricted to luminal membranes of intestinal epithelial cells and robustly amplified in primary and metastatic colorectal tumors. Dysfunction of GCC signaling, reflecting the universal silencing of endogenous paracrine hormone expression early in colorectal tumorigenesis, can be simply restored by oral delivery of GCC ligands with activities compartmentalized to intestine and primary intestinal tumors. These considerations suggest that hormone replacement targeting GCC might be uniquely well qualified for chemoprevention of colorectal cancer in the absence of collateral tissue damage.

Acknowledgements

These studies were supported by grants from NIH (CA75123, CA95026, CA146033), the Pennsylvania Department of Health, and Targeted Diagnostic and Therapeutics Inc., S.A.W is the Samuel M.V. Hamilton Endowed Professor.
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