C-Myc Is a Critical Mediator of the Phenotypes of Apc Loss in the Intestine

Julie A. Wilkins and Owen J. Sansom
Beatson Institute of Cancer Research, Garscube Estate, Swithback Road, Glasgow, United Kingdom

Abstract
The Adenomatous polyposis coli (Apc) gene is mutated in up to 80% of sporadic colorectal cancers. After Apc loss, there is deregulation of the Wnt signaling pathway and transactivation of T-cell factor/leukemia enhancing factor target genes such as C-Myc. This review focuses on recent data highlighting the importance of the C-Myc oncoprotein and its transcriptional targets in establishing all of the phenotypes caused by the deletion of the Apc tumor suppressor gene within the intestinal epithelium. The importance of investigating Apc and C-Myc gene function in the correct tissue context is also discussed. [Cancer Res 2008;68(13):4963–6]

Background
Germline mutation of the APC gene characterizes familial adenomatous polyposis, which is an autosomal syndrome characterized by multiple colorectal lesions at an early age (1). Apc gene mutation also occurs at a very high frequency in sporadic colorectal cancer, where it acts as an initiating event. APC forms part of a destruction complex along with Axin, glycogen synthase kinase 3 β, and caesin kinase 1. This complex promotes phosphorylation of β-catenin, which leads to ubiquitination, followed by proteasomal degradation of the ubiquinated β-catenin (2). When Apc is mutated, the destruction complex is dissociated, leading to an accumulation of free β-catenin, which translocates to the nucleus and activates TCF/LEF (Wnt) target genes.

Apc gene mutation is rare in cancer outside the colon, and recent work deleting the Apc gene in intestinal epithelium (and other Wnt signaling components) have begun to address why the Apc gene acts as a “gatekeeper” against colorectal cancer. Using Cre-Lox technology, two groups have deleted Apc conditionally from the adult murine intestinal epithelium with very similar results (3, 4). Deletion of the Apc gene causes a rapid phenotype. Intestinal crypts become enlarged as intestinal enterocytes continue to proliferate independent of position, fail to differentiate, and no longer migrate up the crypt–villus axis. Thus, Apc-deficient cells can be described as having a “crypt progenitor cell–like phenotype.” Mechanistically, the onset of this phenotype correlates with the nuclear accumulation of β-catenin and the activation of Wnt target genes such as C-Myc (5) and CD44 (6).

C-Myc is a proto-oncogene that has been identified as a Wnt target gene (5) in colorectal cancer cell lines in vitro (7) and in intestinal epithelial crypts after conditional deletion of Apc in vivo (3). The primary function of C-Myc seems to be as a transcriptional regulator that controls up to 15% of the genes in the genome (8). Both genomic and functional analyses of C-Myc targets suggest that C-Myc behaves as a global regulator of transcription; groups of genes involved in apoptosis, cell cycle regulation, metabolism, ribosome biogenesis, protein synthesis, and mitochondrial function are overrepresented in the C-Myc target gene network (8). C-Myc also acts as a transcriptional repressor of genes involved in cell adhesion and growth arrest such as the cyclin-dependent kinase inhibitors p21CIP1 and p15INK4B (8, 9). Through its role as a transcriptional regulator, C-Myc has been shown to regulate numerous critical processes including apoptosis, proliferation, cell metabolism, DNA repair, and angiogenesis. Moreover, recent data have suggested that C-Myc may play a direct role in DNA replication (10). Given this plethora of functions that C-Myc can play in vitro, it is therefore important to translate them to an in vivo setting to assess which of these processes (and transcriptional targets) will be important in a tissue-specific context. The intestinal epithelium is a perfect system for doing this as C-Myc is expressed in the normal intestinal epithelium (within the dividing intestinal crypts) and is overexpressed after Apc loss.

The role of C-Myc in intestinal homeostasis has been a subject of debate. Bettess and colleagues (11) showed C-Myc to be essential for intestinal crypt formation but not for intestinal homeostasis (11). However, Muncan and colleagues (12) showed that C-Myc–deficient intestinal enterocytes had reduced levels of proliferation and were smaller when compared with wild-type intestinal enterocytes (12). Mechanistically, it was proposed that this was due to the reduced levels of the C-Myc target nucleophosmin, which is a key regulator of ribosome biogenesis and cell growth (12). Over the longer term, C-Myc–deficient enterocytes were lost and were replaced with wild-type cells; a phenomenon that can be compared with Drosophila cell competition. Although the reasons for the differences of these two studies are still unclear, it may be due to the fact that different cause recombination (Cre) transgenes were used (AhCre and VillinCreER) and mice were of different genetic backgrounds. These differences are currently being addressed. However, both of these studies showed that C-Myc–deficient cells are viable (albeit over the short term), and thus, the importance of C-Myc for the immediate phenotypes of Apc loss could be assessed.

Key Findings
In our study, we simultaneously deleted both Apc and C-Myc in the murine adult small intestine and analyzed the immediate phenotypes of Apc loss. This was done by intercrossing mice bearing conditional loxP (locus of recombination) flanked knockout alleles for Apc (Apc<sup>lox</sup>) and C-Myc (C-Myc<sup>lox</sup>) with AhCre transgenic mice (3, 13). The ArylHydrocarbon-Cre Recombinase (AhCre) transgene (also known as CYP1A1Cre) delivers near-constitutive expression of Cre recombinase in the intestine.
Figure 1. The relationship between Apc loss, C-Myc activation, and cell fate in a number of epithelia. A, C-Myc is essential for the phenotype of Apc loss in the intestine. Model showing the importance of C-Myc for the intestinal phenotypes of Apc loss. After Apc loss, there is an induction of nuclear β-catenin that results in an up-regulation of two sets of Wnt target genes: those that are C-Myc dependent and those that are C-Myc independent. Despite nuclear β-catenin, the C-Myc–independent wnt target genes are not sufficient to induce any of the phenotypes of Apc loss. This is consistent with the finding that knockout of the Myc-independent Wnt target gene CD44 (a cell surface glycoprotein) does not modify tumorigenesis in the ApcMin/+ mouse (31). Further study of the C-Myc–dependent Wnt target genes should allow us to dissect out which of these genes are important for the phenotypes of Apc loss, namely proliferation, differentiation, and apoptosis. B, overview of Apc loss and Myc expression in other epithelia. Within pancreatic acini (although not islets), Apc loss leads to proliferation that is completely dependent on the expression of C-Myc. Within the renal and mammary epithelium, Apc loss despite nuclear β-catenin and C-Myc expression is not sufficient to drive proliferation and tumorigenesis. Within the liver, Apc loss leads to hyperproliferation and a loss of liver zonation; however, as yet, the functional significance of Myc up-regulation is unknown.
and liver after injection of a cytochrome p450 inducer (13). Remarkably, deletion of C-Myc rescued all the immediate phenotypes of Apc deficiency. Therefore, doubly mutant cells now proliferated, differentiated, and migrated as normal wild-type intestinal enterocytes (14). These effects were not due to loss of doubly deficient cell by apoptosis and overgrowth by wild-type cells, as genomic analyses showed that both genes were gone and, importantly, β-catenin was localized in the nuclei of these cells. The finding that after Apc loss, nuclear β-catenin was not sufficient to induce any phenotypes in the absence of C-Myc was surprising. Importantly, this allowed us to assess, by expression profiling, which Wnt targets are deregulated by Apc loss and which of these are dependent on C-Myc.

Overall, over half of the known Wnt target genes significantly induced after Apc loss were no longer up-regulated in the absence of C-Myc and globally of all the genes up-regulated after Apc loss in vivo 1/3 required C-Myc expression. These data therefore raises the possibility that the phenotypes of Apc loss (in the intestine) might be able to be explained molecularly in terms of C-Myc target gene expression (see Fig. 1A). One of the best examples of this is the expression of the EphB receptors, which control intestinal epithelial architecture and cell positioning through repulsive interactions with Ephrin-B ligands. It has previously been shown that the overexpression of the Wnt target genes EphiB2 and EphiB3 after Apc loss (15) results in mislocalisation of Paneth cells (3). In the double mutants, the EphB receptors are no longer transcriptionally elevated, and a normal localization of the Paneth cells is observed. Another potential C-Myc–dependent Wnt target, which might explain the failed migration of Apc-deficient cells up the crypt-villus axis, is tumor invasion and metastasis 1 (Tiam1). Overexpression of Tiam1 has previously been shown to increase the number of adherens junctions (which are also increased upon Apc loss; ref. 3). Recently, genetic knockout of Tiam1 has been shown to slow intestinal tumorigenesis within the ApcMin/+ mouse, although increase invasion, consistent with the hypothesis that Tiam1 may be very important for the lack of migration observed (16). From the transcriptome analysis, we also observed an induction of genes repressed by C-Myc such as P21. These genes may also play a key role in suppressing the proliferation induced after Apc loss, and current experiments are being undertaken using triple knockouts to assess the significance of p21 up-regulation in cells lacking both Apc and C-Myc.

Implications and Questions Raised

Overall, our data shows that C-Myc is essential for the "crypt progenitor cell like" phenotype of Apc-deficient cells in vivo. At this stage, it is not possible to say whether C-Myc is sufficient for all the phenotypes after Apc loss as this would require exactly the same level of C-Myc to be overexpressed that occurs after Apc loss, and this currently is not technically possible (8, 17–19).

One of the central questions that remain is how tissue/context specific this finding will be (see Fig. 1B). This is perhaps best answered by examining the effect of Apc deficiency in other tissues. In many other cancers, Wnt signaling is often deregulated, yet this is not thought to be the initiating event. Modeling loss of Apc in other tissues has begun to explain this phenomenon. Within both the developing mammary gland and the renal epithelium, Apc loss alone provides a selective disadvantage where cells undergoing pre-meditated recombination and Apc deletion being lost (20, 21), whereas loss of Apc in the developed mammary leads to transdifferentiation to squamous metaplasia, which is resistant to undergoing transformation (20). In these scenarios, Apc is lost, C-Myc is up-regulated, and yet the cells do not have a selective advantage, underscoring that the precise cellular context is crucial to the outcome an Apc mutation. In these tissues, other mutations such as P53 loss or K-Ras activation are required before tumorigenesis can ensue, and it will be of great interest to assess whether these tumors are dependent on C-Myc expression (22).

A recent study removing Apc in the pancreas again highlights the importance of cellular context (19). Given the seminal studies of C-Myc overexpression in the pancreatic islets (which leads to a large induction of proliferation and apoptosis; ref. 23), one would have expected Apc loss within pancreatic islets to lead to a dramatic phenotype if C-Myc was overexpressed. However, when Apc was deleted within the islets of the pancreas using PA6X-Cre, despite efficient gene deletion, no phenotype was observed. This lack of phenotype correlated with a complete failure to see activation of Wnt/Tcf target gene expression after Apc loss such as C-Myc. The finding that Apc can be lost without target gene expression was a remarkable finding, as dogma would suggest that this would never occur. Using the pancreatic and duodenal homeobox Cre recombinase to drive recombination throughout the entire pancreas, Apc gene deletion led to pancreatomegaly, due to the hyperproliferation of acinar cells, although not tumorigenesis. Coinactivation of C-Myc led to a complete block of the pancreatomegaly and the acinar hyperplasia, indicating the possibility that Wnt-mediated hyperplasia may be dependent on C-Myc in a number of different epithelia. However, until this is tested, one should take great care in generalizing both the effect of Apc loss and the requirement of C-Myc. For example, a predicted consequence of Apc loss in the liver is hepatomegally and hyperproliferation, which is observed, although few would have predicted that Apc would play a role in liver zonation (24). It should also be noted that a number of recent studies have shown that Wnt signaling is activated during liver regeneration, and β-catenin is required for efficient regeneration. However C-Myc loss does not impair liver regeneration (25). This could suggest that Wnt-dependent proliferation within the liver may not be dependent on C-Myc, although this still needs to be formally tested. Also, it possible in certain epithelia other Myc family members such as N-Myc or L-Myc may be crucial downstream of Wnt signaling.

Finally, the finding that C-Myc is a central mediator of the Apc deficiency within the intestine raises the possibility that C-Myc inhibition may be a good target for chemoprevention of colorectal cancer. This possibility is already well-supported in the literature as both haploinsufficiency for c-Myc and complete loss of c-Myc modifies intestinal tumor formation in ApcMin/+ mice (26, 27). It is interesting to note that genetic knockout of the C-Myc target genes Mif (28) or Tiam1 (16) slows adenoma formation in the ApcMin/+ mouse, and further investigation of C-Myc–dependent target genes after Apc loss may identify numerous other modifiers of intestinal tumorigenesis. It will also be of great interest to investigate whether C-Myc inactivation in murine intestinal adenomas and adenocarcinomas will cause tumor regression as has been seen in other tumor types in vivo (29, 30).

In summary, our work has identified the C-Myc network as the key downstream pathway after Apc loss within intestinal
epithelium. Future work in vivo is required to define the functional importance of these C-Myc–dependent targets in the intestine after Apc loss. Future work will also be required to assess whether C-Myc is a critical mediator of the Apc loss in other epithelia. Given that Apc-deficient cells are ”addicted” to C-Myc within the intestinal epithelium, this raises the possibility that inhibition of C-Myc may be a viable approach for chemoprevention of colon cancer, and further elucidation of the C-Myc pathway in these cells may yield many novel modifiers of intestinal tumorigenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 9/20/2007; revised 2/23/2008; accepted 3/12/2008.

References

C-Myc Is a Critical Mediator of the Phenotypes of Apc Loss in the Intestine

Julie A. Wilkins and Owen J. Sansom


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/13/4963

Cited articles
This article cites 31 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/13/4963.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/68/13/4963.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.