Giving *APCmin* tumours a SPARC

Alex Gregorieff, Hans Clevers

A new study identifies the extracellular matrix (ECM) component, SPARC (secreted protein acidic, rich in cysteine), as a critical determinant of tumour burden in the *APCmin*/+ model of intestinal cancer.

The genetic lesions that initiate colorectal cancer are relatively well understood. In the overwhelming majority of cases, colorectal cancer cells carry mutations in components of the Wnt signaling pathway (most often in the adenomatous polyposis coli (*APC*) gene), which confers upon them a marked growth advantage over surrounding normal epithelial cells.^{1,2} Although this initial burst in proliferative potential represents an important event in the progression towards adenomas and later carcinomas, clearly other contributing factors must also play their part. Over the years, the classical *APCmin*/+ model of intestinal tumorigenesis has provided researchers with an excellent tool to test genetically whether candidate genes can enhance or repress adenoma formation *in vivo*.^{3,4} Phenotypically, *APCmin* mice resemble familial adenomatous polyposis patients, in that they carry inactivating mutations in the *APC* gene and spontaneously develop numerous polyps along their intestinal tract.⁵

MODIFYING THE MIN PHENOTYPE

The number of *APCmin* tumours is strongly influenced by the genetic background. On the basis of this observation, linkage studies have identified loci that modify tumour load in *APCmin*/+ mice, most prominent of which is the secretory phospholipase A2 (*Pla2g2a*).⁶⁻⁹ A more effective strategy for identifying modifiers has, however, come through candidate approaches. For a start, several laboratories have looked at the role of genetic surveillance factors such as Mbd4,¹⁰ Myh,¹¹ Fen1¹² and Msh2.¹³ These proteins help repair DNA errors, and by doing so, reduce the risk of potentially tumorigenic mutations from occurring. Accordingly, the inactivation of such genes results in accelerated polyp formation in *APCmin*/+ mice. Another way to augment tumorigenicity is through aneuploidy, a consequence of missegregated chromosomes during cell division. One study has indeed shown that loss of a single allele of the spindle checkpoint component, BubR1,

causes greater numbers of colonic adenomas with enhanced genomic instability,¹⁴ but paradoxically BubR1 deficiency also led to lower polyp counts in the small intestine. Similarly, the decreased expression of the transcription factor Cdx2 in *APC*-deficient mice is also associated with higher rates of chromosomal aberrations and increased colonic polyp numbers, whereas in the small intestine adenoma formation was reduced.¹⁵

Another important class of modifiers of the *APCmin* phenotype constitutes genes that are transcriptionally regulated by the Wnt signaling pathway (see table 1). Inactivating the *APC* gene leads to a robust increase in β -catenin/Tcf transcriptional activity.¹⁶ In recent years hundreds of genes regulated by β -catenin/Tcf have been reported, some of which can modulate adenoma formation (a partial list includes cMyc^{17,18}, EphB2/B3,¹⁹ Tcf1,²⁰ Tiam1,²¹ and Cox2²²). For an updated list of Wnt target genes see <http://www.stanford.edu/~rnusse/wntwindow.html>. Interestingly, certain Wnt target genes (i.e. *EphB* receptors and *Tiam1*) stimulate the invasiveness of adenomas upon inactivation.^{21,23} It is worth noting here that the progression of *APCmin* adenomas to adenocarcinomas is also associated with parallel signaling cascades such as those regulated by Ras and Tgf β .^{24,25}

The regulation of gene expression on a more general level also plays a role in intestinal neoplasia. For example, deficiencies in global regulators of transcription such as the histone deacetylase, HDAC-2;²⁶ the DNA methyltransferase, Dnmt1;²⁷ and the methyl binding domain protein, Mbd2,²⁸ all result in impaired tumorigenesis in the *APCmin* background. A similar reduction in *APCmin* tumours has been noted in mice lacking the AP-1 component, c-Jun,²⁹ and the partner of p120-catenin, Kaiso.³⁰ In the case of c-Jun, the suppression of adenoma formation may be caused by the ability of c-Jun to promote β -catenin/Tcf activity. Finally, a more specialized transcription factor such as FoxL1 has the opposite effect.³¹ The deletion of *FoxL1* promotes tumour

formation in the intestine and also initiates gastric tumours. The fact that FoxL1 is expressed in stromal cells suggests that it might regulate the expression of secreted factors that favour tumour development in a paracrine fashion.

THE ECM COMPONENT SPARC PROMOTES ADENOMA FORMATION

The interface between tumour cells and the extracellular matrix (ECM) has long been appreciated as an important determinant of tumour progression.³⁵ Note that the ECM in this context simply refers to all material constituents surrounding any given cell. The first ECM component linked to tumorigenesis in the *APCmin* model was the matrix metalloproteinase and Wnt target gene, MMP7/Matrilysin.³³ In this issue of *Gut*, Owen Sansom and colleagues identified SPARC (secreted protein acidic, rich in cysteine), as a novel ECM factor involved in adenoma formation. Their results show that SPARC protein levels are upregulated in polyps arising in *APCmin*/+ mice and in human colorectal adenocarcinomas. The significance of these findings was confirmed by demonstrating that the removal of SPARC in *APCmin*/+ mice suppressed the multiplicity of adenomas, while not affecting the size of tumours. Finally, the authors also measured migration rates of epithelial cells and found that SPARC-deficient cells transited more rapidly up the crypt-villus axis, a result implying higher turnover rates of the intestinal epithelium.

SPARC is a so-called matricellular protein and is part of a family of structurally unrelated extracellular modulators of cellular function.³⁶ Members include thrombospondin 1 and 2, osteopontin, cyr61, CTGF, CCN1 and the tenascins. The distinguishing feature of this diverse group is that they are all secreted macromolecules, which appear not to serve any structural roles. Instead, matricellular proteins regulate cell-matrix interactions, bind numerous constituents of the ECM and are generally involved in modulating adhesion.

The biological function of SPARC has been studied in cultured cells as well as in knockout mice. *In vitro* SPARC promotes de-adhesion, a phenomenon characterized by the transition between a state of strong to intermediate adhesion, accompanied by a restructuring of focal adhesions and stress fibres.³⁷ SPARC null mice display altered responses to cutaneous wounds as well as varied developmental defects affecting the formation of the lens capsule, dermis, adipocytes, and bone.³⁸ SPARC mutant mice have also been useful in establishing a role for SPARC in

Table 1 Summary of genes that modify intestinal adenoma formation resulting from deletion of the APC gene

Gene	Effect on tumour formation	Reference
DNA repair/chromosome instability		
Mbd4	+	Millar <i>et al</i> ¹⁰
Myh	+	Sieber <i>et al</i> ¹¹
Fen1	+	Kucherlapati <i>et al</i> ¹²
Msh2	+	Reitmair <i>et al</i> ¹³
BubR1	(see text)	Rao <i>et al</i> ¹⁴
Cdx2	(see text)	Aoki <i>et al</i> ¹⁵
Wnt target genes		
c-Myc	–	Sansom <i>et al</i> ¹⁷ , Ignatenko <i>et al</i> ¹⁸
EphB2/B3	(+ invasiveness)	Battile <i>et al</i> ¹⁹
Tiam1	(+ invasiveness)	Malliri <i>et al</i> ²¹
Tcf1	+	Roose <i>et al</i> ²⁰
Cox2	–	Oshima <i>et al</i> ²²
Transcriptional regulators		
HDAC2	–	Zhu <i>et al</i> ²⁶
Dnmt1	–	Eads <i>et al</i> ²⁷
Mbd2	–	Sansom <i>et al</i> ²⁸
Kaiso	–	Prokhortchouk <i>et al</i> ²⁹
cJun	–	Nateri <i>et al</i> ²⁹
FoxL1	+	Perreault <i>et al</i> ³¹
Miscellaneous		
Pla2g2a	(see text)	Dietrich <i>et al</i> ⁶ , McPhee <i>et al</i> ⁷
Ras	(see text)	Sansom <i>et al</i> ²⁵
Smad4	(see text)	Alberici <i>et al</i> ²⁴
PKCalpha	+	Oster and Leites ³²
MMP7/Matrilysin	–	Wilson <i>et al</i> ³³
SPARC	–	Sansom <i>et al</i> ^{34*}

Genes marked with a (+) promote adenoma formation upon deletion and genes marked with a (–) suppress adenoma formation upon deletion. For coherence, genes are subdivided into four functional groups (see text for details).

*See p1410 this issue.

neoplasia. In brief, the loss of function of SPARC seems to have disparate effects depending on the cellular context. For example, the subcutaneous injection of tumour cells in SPARC-deficient mice resulted in enhanced tumour growth, with evidence of reduced collagen deposition and macrophage infiltration.³⁹ Whereas in a mammary carcinoma model, tumour size, as well as collagen deposition and vascularization were reduced in SPARC^{–/–} mice.⁴⁰

What do all these studies tell us about the function of SPARC in intestinal neoplasia? In a system with a high turnover rate, such as the intestinal epithelium, tumour cells must find a way to embed or seed themselves into the mucosa in order to avoid the general flux that pushes enterocytes out into the lumen. It has been argued that SPARC and probably other ECM components might be essential in this 'seeding' effect.³⁶ In other words, through SPARC's ability to regulate processes such as the deposition of ECM components and vascular growth and remodeling, SPARC might be essential in preparing the stromal compartment in the intestine for efficient tumour growth and invasion. An alternative explanation may depend on the recognized role of SPARC in regulating adhesion. Indeed, Sansom and colleagues propose that the higher migration rates of SPARC^{–/–} enterocytes

may contribute to eliminating deleterious cells and thus suppressing adenoma formation. Finding out what precisely SPARC does during intestinal tumorigenesis will be the next challenge.

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Colorectal cancer

What is the role of iFOBT in screening for colorectal cancer?

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Policy makers will need to consider if it has one, not only as an adjunct to gFOBT screening, but also as a primary screening test

Screening for colorectal cancer (CRC) using gFOBT (guaiac based faecal occult blood test) has been shown in randomised controlled trials (RCTs) to reduce CRC mortality.^{1–3} gFOBT testing is endorsed as an option for CRC screening in the United States^{4–7} and is being implemented in the United Kingdom. People with a positive gFOBT receive colonoscopy to detect early cancers and advanced adenomas that, if untreated, might cause CRC mortality. Because gFOBT has a high rate of false positive results, however, gFOBT screening can incur substantial cost and use of colonoscopy resources. A method that could determine which people with a positive gFOBT have false positive results—and do not need colonoscopy—would make gFOBT screening more practical.

A study in this issue of *Gut*⁸ Fraser *et al* (see p 1415) shows that doing iFOBT (human haemoglobin immunochemical based FOBT) in people with a positive gFOBT will detect almost all clinically important lesions in gFOBT positive individuals, while reducing false positive results and the need for colonoscopy. In this study, both iFOBT and colonoscopy were done in those with a positive gFOBT, defined as one to four positive gFOBT ovals (people with five or six positive ovals were automatically referred for colonoscopy). The sensitivity of iFOBT among people with positive gFOBT was 95.9%, while specificity was 59.2%, resulting in a 30% reduction of colonoscopy use. The mechanism by which iFOBT achieves higher specificity than gFOBT

presumably is elimination of false positives that occur in guaiac based testing from sources other than human haemoglobin, like diet.

Learning that iFOBT may have a role in a programme of gFOBT screening is practical and important, but it raises a larger question that will need to be sorted out over time. Should iFOBT replace gFOBT as the primary test in a programme of FOBT screening? The authors note that the unit cost for iFOBT is higher than for gFOBT, but that is only one consideration.⁸ Others include the increased benefit that might be achieved, additional costs (including false positives), and the public's willingness to pay for greater benefit.

The potential usefulness of iFOBT vs gFOBT screening depends largely on the “absolute sensitivity” of each FOBT, a question not addressed in this study that assessed iFOBT sensitivity only among those who already had a positive gFOBT. Used in this way, iFOBT can never do better than gFOBT.

What is the absolute sensitivity for each FOBT? Obtaining absolute sensitivity for any FOBT is logistically difficult, requiring administration of both the FOBT and a “gold standard” exam (like colonoscopy). In asymptomatic individuals the prevalence of CRC is so low, roughly 1–3 per 1000, that thousands of people need to be studied. In one report, gFOBT sensitivity for CRC was about 30%; in another it was 13%.¹⁰ The different results might be explained by use of centralised FOBT processing in the

former study (as is also done in the current study, in RCTs, and in the UK screening programme being implemented), while in the latter study processing was done in each physician's office.

While these are low values for absolute sensitivity of gFOBT, they are high enough, as shown in RCTs, to reduce CRC mortality by 33% in a US¹ trial and by about 16% in two European trials.^{2–3} Some mortality reduction may be achieved by repeated application of FOBT in a programme of screening. gFOBT screening is considered not only an “option” for CRC screening, but when combined with sigmoidoscopy, it may be competitive, in terms of effectiveness, with a programme of colonoscopy screening.^{11–12} This seeming paradox—that a less sensitive test like FOBT might be as effective or more effective than a “gold standard” test like colonoscopy—can be explained by considering how a “programme” of screening works. If a test with lower sensitivity at any one application is applied repeatedly over time in a programme of screening, it may result in a higher “programme” sensitivity than when a more sensitive test (like colonoscopy) is applied less frequently, because a more frequently applied test can detect fast growing CRC that would be missed by a less frequently applied test.^{11–13}

If gFOBT is not simply “acceptable” but perhaps is even competitive with colonoscopy in some settings, then what would be the impact of a better FOBT? The absolute sensitivity of iFOBT in recent studies has been shown to be roughly 60% for CRC,^{14–17} much higher than for gFOBT. Even without new RCT data to assess CRC mortality reduction, policy decisions may be based on data about the sensitivity and specificity of newer tests, existing RCT results, and modelling.⁷ Policy makers will need to consider whether iFOBT has a role not only as an adjunct to gFOBT screening, but also as a primary screening test.

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