Mouse models of intestinal cancer

Rene Jackstadt and Owen J Sansom*

Cancer Research UK Beatson Institute, Glasgow, UK

*Correspondence to: OJ Sansom, Cancer Research UK Beatson Institute, Switchback Road, Garscube Estate, Glasgow G61 1BD, UK. E-mail: o.sansom@beatson.gla.ac.uk

Abstract

Murine models of intestinal cancer are powerful tools to recapitulate human intestinal cancer, understand its biology and test therapies. With recent developments identifying the importance of the tumour microenvironment and the potential for immunotherapy, autochthonous genetically engineered mouse models (GEMMs) will remain an important part of preclinical studies for the foreseeable future. This review will provide an overview of the current mouse models of intestinal cancer, from the ApcMin/+ mouse, which has been used for over 25 years, to the latest ‘state-of-the-art’ organoid models. We discuss here how these models have been used to define fundamental processes involved in tumour initiation and the attempts to generate metastatic models, which is the ultimate cause of cancer mortality. Together these models will provide key insights to understand this complex disease and hopefully will lead to the discovery of new therapeutic strategies.

Keywords: colorectal cancer; crc; invasion; metastasis; transplantation; GEMM; adenomatous polyposis coli; organoids

Received 3 September 2015; Revised 21 September 2015; Accepted 23 September 2015

No conflicts of interest were declared.

Introduction

In the Western world, colorectal cancer (CRC) is the second-highest cause of cancer mortality [1]. In ~90% of fatal cases, metastasis is the cause of mortality. In the early 1990s Fearon and Vogelstein [2] postulated that mutations in CRC occur in a sequential manner, with specific mutations being associated with tumour initiation, eg the adenomatous polyposis coli (APC) gene, and other mutations occurring later that drive progression, eg TP53. Recent DNA sequencing studies confirmed the common co-existence of these mutations within individual CRC tumours. A recent theory of CRC, referred to as the ‘Big Bang’ model, describes tumour growth as an expansion populated by various heterogeneous subclones. Initial mutations in genes, such as APC and KRAS (‘public mutations’), are carried by all subclones, and subsequent ‘private’ mutations are acquired later in individual subclones [3].

In 80–90% of CRCs the initial step is proposed to be the loss of the tumour-suppressor gene APC, and this is often called the ‘classical’ route [4]. Inactivation of APC induces stabilization of β-catenin (as it can no longer be targeted for degradation) and translocation of β-catenin to the nucleus. In the nucleus β-catenin acts as a transcriptional co-activator, interacting with TCF4/LEF1 transcription factors to up-regulate expression of WNT target genes [4,5]. Another early event during tumour progression is the mutation of the proto-oncogene KRAS. KRAS is mutated in 40–50% of human CRCs, with >75% of these mutations located in codon 12, which lock KRAS in the active GTP-bound state [6].

Further common mutations occur to activate the PI3 kinase signalling pathway, eg in PTEN or PIK3CA. This pathway is associated with driving cell growth, metabolism and tumour progression. TGFβ pathway abrogation in CRC can occur through mutation of either TGFβ-receptor 1 (TGFBR1) or TGFBR2. Furthermore, TGFβ pathway inactivation can occur via loss of heterozygosity (LOH) of chromosome 18q, where SMAD2 and SMAD4, two downstream mediators of TGFβ signalling, are located. Another gene deleted in colorectal cancer (DCC) is also localized to 18q and encodes a netrin receptor that controls differentiation and tumourigenesis [7,8]. A further late-stage event, mainly associated with tumour cell invasion, is the mutation of the tumour-suppressor gene TP53 [6]. Interestingly, tumours carrying TP53 and APC mutations are often associated with increased rates of chromosomal instability (CIN) [9–11].

Sequencing studies have also revealed that many other mutations occur in individual CRC tumours, although at much lower frequencies (the ‘private’ mutations described above). The importance of these is still unclear and many represent passenger mutations which might have no function [12–14]. Mouse models still provide the ‘gold standard’ test to see whether these mutations can functionally affect the development of cancer.

Of the remaining 20% of CRC tumours that do not carry APC mutations, many of these are associated with...
mutation of DNA mismatch repair (MMR) genes or inactivation predominantly of the mismatch repair genes MLH1 and MSH2 (Lynch syndrome) [15–17]. These cancers have very high levels of mutation rate, evidenced by high levels of microsatellite instability, and are predominantly right-sided and carry an improved prognosis. Recently an excellent model of Lynch syndrome has been developed through targeted deletion of Msh2 in the intestinal epithelium [18]. The mutational spectra havelimitedourreviewtogeneticmodelsofcancerand bethefocusofthisreview.Duetospacelimitations,we arebasedonthegeneticmake-upoftumours,generating realistic mouse models of the human disease. The successes and challenges that still need to be overcome will be the focus of this review. Due to space constraints, we have limited our review to genetic models of cancer and so do not discuss colitis-associated cancer models within the mouse (reviewed in [20,21]). A brief overview of the models discussed in this review is provided in Table 1.

Mutation of APC leads to adenomas in mice

APC loss is the cause of familial adenomatous polyposis (FAP), a human autosomal dominant syndrome, in which patients develop numerous colorectal polyps [22,23]. Given the high prevalence of APC mutation in sporadic colorectal cancer and APC being the causal gene for FAP, most of the models developed to mimic

| Model | Invasion | Metastasis | Reference |
|-------|----------|------------|-----------|
| Apc^1838N/+ | Increased mucosal and submucosal invasion | Liver metastasis (1) | Fodde [42] |
| AhCre Apc^fl/+ Kras^{G12V} | 17% invasive carcinoma, into smooth muscle | | Sansom [44] |
| AhCre Apc^fl/+ Pten^fl/fl | 32% early invasive adenocarcinomas; 22% advanced adenocarcinomas | | Marsh [77] |
| Fob1Cre Pik3ca^+/+ | Invasive adenocarcinoma (analysed at day 40) | | Leystra [78] |
| Fob1Cre Pik3ca^+/+ Apc^Min/+ | Invasive adenocarcinoma | | Deming [79] |
| VillinCre Apc^1838N/+ Tgfbr2^fl/fl | 41% invasive carcinoma | | Munoz [81] |
| Apc^fl/+ Smad4^−/+ | 54% submucosal invasion | | Takaku [82] |
| Apc^fl/+ Smad3^−/+ | Invasion to submucosa and into the muscularis propria | | Sodiri [83] |
| Apc^fl/+ Smad2^−/+ | 10–15% stroma invasion | | Hamamoto [84] |
| Apc^Min/+ p53^−/+ | Muscularis mucosa | | Halberg [87] |
| AhCre Apc^fl/+ p53^fl/fl | 25% stromal invasion | | Muller [88] |
| AhCre Apc^fl/+ p53^{R172H}/fl | 100% stromal invasion | | Muller [88] |
| Apc^Min/+ Δn EphB2 | 100% (>30 tumours from seven different mice) classified as intramucosal adenocarcinomas | | Battie [90] |
| Apc^Min/+ EphB2^−/+ | 47% of the tumours were scored as invasive carcinoma | Metastasis to the mesenteric lymph nodes in 20% (1/5) of the mice | Rad [113] |
| VillinCre Braf^FLSL−/V637E/+ | 14% (4/29) of mice of mice showed invasive carcinoma at age 10 months | Metastasis to the lung, pancreas, liver and mesenteric lymph nodes in 25% (3/12) of the mice | Rad [113] |
| VillinCre Braf^V637E/+ p53^{R172H}/+ | 56% (10/18) of mice showed invasive carcinoma at age 10 months | Metastasis to the lung, stomach, liver and local lymph nodes in 25% (3/12) of the mice | Rad [113] |
| VillinCre Braf^V637E/+ p16^−/+ | 59% (20/34) of mice of mice showed invasive carcinoma at age 10 months | | |
| VillinCreEr22 Apc^fl/+ Pten^fl/fl Kras^{G12V}/+ | High-grade invasive carcinoma in 7% of the tumours | 41% (n = 11/27) present metastases; liver (7/11), pancreas (3/11), lymph nodes (2/11) and lungs (1/11) | Davies [114] |
| VillinCreEr22 Pten^fl/fl Kras^{G12V}/+ | 44% (12/27) showed invasion into the intestinal wall | | Davies [114] |
| VillinCre Kras^{G12V}/+ Tgfbr2^fl/fl | 70% showed marked desmoplasia and invasion | Lymph node and lung metastasis in 15% (3/20) | Trobridge [115] |
| VillinCre Kras^{G12V}/+ Ink4a/Arf^−/+ | Serrated invasive carcinoma in 76% (13/17) | Metastasis to the lung in 62% (6/10) of mice with invasive carcinoma | Bennecke [116] |
| VillinCreEr22 Nicd^L124/+ p53^fl/fl | 59% showed invasion into muscularis and adipocyte tissue | 23% (n = 7/30) lymph node and 10% (n = 3/30) liver metastases | Chanrion [121] |
Figure 1. Timeline of the development of murine intestinal cancer models. The Apc<sup>Min/+</sup> mouse was developed in 1990 and recapitulates the disease observed in FAP patients. In 1997, the first conditional deletion of Apc was performed in the colon and led to colonic adenomas. Acute deletion of Apc throughout the intestine led to a crypt progenitor phenotype in which whole crypts were transformed. To model more advanced disease, the Apc<sup>780S</sup> (and Apc<sup>Min/+</sup>) model was combined with commonly mutated oncogenes/tumour suppressor-related genes (2000 onwards). This led to faster tumourigenesis and to increased penetrance of invasive adenocarcinomas but not metastasis. With more interest in serrated models of CRC, models driven by Kras or Braf mutations were generated. These models lacked Apc mutation and tumour latency was much increased. However, these models commonly generated adenocarcinoma that had the capacity to metastasize. Most recently, tumour-derived and primary organoids transformed with common CRC mutations have been implanted into syngeneic or immunocompromised mice, either subcutaneously or into the kidney capsule (2015). Metastasis has been observed from tumour-derived organoids.
Functional genetic studies have identified numerous genes that modulate tumour development by both acceleration and deceleration. Initial studies identified modifier of MIN (MOM) loci through genetic linkage studies in mice. MOM1 is located distal to chromosome 4. Interestingly, the orthologous region on the human chromosome shows frequent LOH in CRC [31]. The two genes located within the mouse MOM1 region are Plag2g2a and perlecan (Hspg2) and studies have identified that disruption of Plag2g2a can slow tumourigenesis [32]. Further modifiers of MIN have been described and reviewed [33,34]. The identification of MOM1 also highlighted the importance of mouse genetic background on tumourigenesis [31,35].

Apc\textsuperscript{Min/+} C57BL/6J mice develop 30 polyps on average. Crossing these mice with AKR, MA or CAST strains dramatically reduces the number of polyps, indicating that the MOM1 locus is lost in C57BL/6J mice [35,36]. This has been tested by introducing distal chromosome 4 from AKR mice into C57BL/6J mice [35]; congenic mice showed the semi-dominant function of the MOM1 locus. Therefore, it is important to analyse Apc\textsuperscript{Min/+} mice in a C57BL/6J background. Otherwise, tumour burden and latency varies strongly, potentially masking the effects of the genes being tested. Many other factors can modify intestinal tumourigenesis in the Apc\textsuperscript{Min/+} mouse, such as diet and the microbiome [37,38]. Recently, novel approaches have been used to discover new modifiers of tumourigenesis in the Apc\textsuperscript{Min/+} mouse; sleeping beauty transposon-mediated mutagenesis identified hundreds of alleles that can accelerate tumourigenesis in this system [14]. One caveat that should be mentioned here is that if the mutation causes late-stage progression, this might not have a phenotype in a model that only predisposes to adenoma.

Given the high penetrance of the APC mutation in human CRC, many other Apc-truncating alleles have been generated. These include an allele, Apc\textsuperscript{1322T/+}, which very closely mimics the mutations that occur in human cancer (APC codon 1309) [39] and an Apc knockout allele that produces no protein [40]. All the alleles that cause a loss of the ability of APC to bind β-catenin lead to intestinal tumour predisposition; however, precise kinetics and tumour features can alter depending on the allele. For example, Apc\textsuperscript{1322T/+} shows increased levels of Lgr5 and stem cell markers within tumours, although with a slight reduction in general Wnt target gene expression, eg Axin2 [41]. Another interesting example of these mutations is Apc\textsuperscript{1638N/+}, which harbours a neomycin cassette in antisense orientation within exon 15, resulting in a protein truncated at codon 1638, which is unstable. These mice show few tumours (<10) and a long latency, and develop adenocarcinoma along with infiltration into the mucosa and submucosa [42]. Thus, mice might develop tumours that more closely resemble human CRC if there were a longer latency to tumour development that allowed them to acquire further mutations that drive progression.

**Spatio-temporal control of gene expression in vivo**

The advent of Cre–lox (Cre) technologies in the 1990s enabled researchers to delete any gene in any tissue of interest [43]. In this method, mice carrying a Cre transgene (under the control of an inducible tissue specific promoter) are crossed to mice bearing an inducible allele where the region that is to be deleted is flanked by LoxP recombination sites. This can be either an essential exon(s) of a gene, to produce a conditional knockout, or a Stop motif to activate an oncogene, eg Kras or Pik3, within adult tissue [44,45]. The inducibility of Cre recombinases was most commonly achieved by coupling the Cre enzyme to the oestrogen receptor, leading to activation of Cre after administration of tamoxifen [46]. Titration of Cre induction either via reducing the inducing agent (tamoxifen/viral) or Cre recombinase also facilitates low levels of recombination, which was hoped to overcome problems of multiple tumours per mouse [47].

**APC deletion**

Acute deletion of both copies of Apc has revealed much about the mechanism of early tumourigenesis. Shibata et al [48] delivered Adenovirus–Cre to the colon and showed that deletion of both copies (LoxP sites flanking exon 14; Apc\textsuperscript{580S/580S, Apc\textsuperscript{6B}}) was sufficient to drive colon adenomas. Using a highly penetrant inducible Cre (AhCre, which is driven by the Cyp1a1 promoter and is inducible by β-naphthoflavone and VillinCre\textsuperscript{ERT}) within the small intestine (and to a lesser extent the colon), we [49] and Andreu et al [50] showed that Apc loss had a dramatic impact on intestinal homeostasis. Deletion of both copies of Apc results in a crypt progenitor phenotype, which is characterized by increased proliferation and altered migration and differentiation. Notably, this phenotype was mediated by the Wnt target gene Myc [51,52]. We and others have identified a number of Wnt-Myc targets important for this [53–55]. More recently, colon-specific deletion of Apc has been achieved using a Cdx2P–Cre\textsuperscript{ERT2} transgenic mouse and produced a very similar phenotype to that of deletion of Apc in the small intestine [56]. Using constitutive or inducible colon-specific Cre also overcomes the problem of small intestinal tumour burden and many different colon Cre (FABPCre, A33Cre, CDX2Cre) have all been used to delete a single copy of Apc and generate colonic adenomas [57–59].

The discovery of LGR5\textsuperscript{+} intestinal stem cells (ISCs) in the small and large intestine not only led to fundamental changes in concepts on ISCs and homeostasis but also allowed us to explore the impact of deleting Apc in the ISCs [60]. LGR5 is a G-protein coupled receptor that binds R-spondin and thereby enhances Wnt signalling [61]. LGR5 was shown to be a ‘bona fide’ ISC marker using lineage tracing. In brief, a knock-in
Lgr5CreER mouse was generated and interbred with the Rosa26SL-LoxZ reporter mouse. Following Cre induction, LGR5 ISCs were able to stably generate all epithelial lineages [60]. Notably, using Lgr5-CreER to delete Apc within ISCs led to rapid formation of intestinal adenomas, strongly suggesting that LGR5+ ISCs might be the cells of origin for intestinal cancer [62]. Following this study, many other stem cell markers have been identified and, using a similar Cre knock-in approach, ISCs have been shown to act as cells of origin for cancer when Apc is deleted or a constitutive-active β-catenin is expressed [63–65]. Together these studies showed in the mouse that ISCs are highly efficient cells of origin for cancer.

However, two studies have recently demonstrated that activation of Wnt signalling in differentiated cells results in dedifferentiation and adenoma formation [66,67]. This dedifferentiation seems to require further events, eg inflammation or another oncogenic event, in addition to deregulation of Wnt signalling. Activation of β-cateninΔex3/Δex4 and the inflammatory nuclear factor-κB (NFκB) signalling pathway, in non-ISCs (using the Xbp1–CreER), led to dedifferentiation and tumour development [66]. The same study demonstrated that concomitant Apc deletion with aberrant KrasG12D/+ expression results again in a NF-κB-dependent dedifferentiation. This observation is in accordance with the ‘top-down’ model of CRC development, which is based on the observation that early dysplastic human CRC lesions predominantly locate to the luminal part and not to the base of the crypt [68]. Another study investigating the potential transformation of differentiated cells targeted Apc deletion to terminal differentiated tuft cells, using a tuft cell marker, DCKL1. Although Dckl1–Cre-mediated loss of Apc alone did not lead to tumour formation, when Apc loss was combined with dextran sodium sulphate (DSS) treatment (to induce colitis) the mice developed tumours [67]. Therefore, these studies show that mouse models can inform us about the capacity of cells to act as cells of origin for cancer. The key question that remains is whether they do so in human cancer. Further cross-comparison with human tumours and mathematical modelling is required for us to progress beyond these ‘proof-of-principle’ experiments.

A fundamental drawback of Cre-mediated gene inactivation is that this results in the permanent deletion of a gene, and thus it is hard to assess the sustained requirement for the initiating oncogene/tumour suppressor gene. To address the continued reliance on Apc loss and downstream Wnt signalling, two recent studies using doxycycline-inducible systems have shown that, if APC expression is restored (through inducible shRNA) or an inducible active β-catenin allele is turned off, there is complete reversion to a normal intestinal epithelium. This underlines the continued dependence on Wnt signalling [31,69]. In all situations, withdrawal of doxycycline led to down-regulation of Wnt signalling and complete tumour ablation via differentiation. This even occurred in invasive adenocarcinomas also carrying mutations in Tp53 and KrasG12D/+ [70]. Therefore, GEMMs of CRC provided excellent ‘proof of concept’ that a target remains important throughout all stages of carcinogenesis.

Generating mouse models of adenocarcinoma carrying Apc mutation

Generating mouse models of metastatic CRC has proved to be difficult. One of the key steps towards modelling metastasis is generating murine models of invasive adenocarcinoma. Cellular invasion is a complex process in which tumour cells escape from the adhesive epithelium and cross the basement membrane, invading the smooth muscle of the intestine. This is often associated with a change in cellular shape, gain of motility and loss of E-cadherin [71]. Single-cell migration can be achieved by epithelial cells which undergo an epithelial–mesenchymal transition (EMT), resembling a developmental process [72]. This process is regulated by intercellular communication of tumour cells with their microenvironment, typically mediated by cell–cell communication via chemokines or the extracellular matrix (ECM) [73]. Notably, EMT has been suggested to be a dominant process during human CRC progression [74].

As mentioned above, CRC progression follows a distinct order of serial mutations [2]. Since Apc mutations alone do not produce invasive tumours, later mutations in the adenoma–carcinoma sequence have been added to make mouse models of CRC more patient-relevant. With a mutation rate of ~40% in human CRC, Kras is one of the most frequently altered genes following APC and is also described as an early event during progression [2]. Mouse models combining mutation of Apc with aberrant expression of mutated KrasG12V/+ resulted in a higher number of intestinal tumours with an increased invasion of tumour cells to the surrounding stroma [44,75]. Given the high frequency of PTEN and PI3KCA mutations in human CRC [76], both Pten and Pik3ca mutant mice have been intercrossed with mice carrying Apc mutation. These additional mutations rapidly accelerate tumourigenesis and increase tumour progression so that the mice develop adenocarcinomas [77]. When active Pik3ca is expressed alone within the intestine, the mice develop invasive mucinous adenocarcinoma with no intermediate benign tumour stage [78]. Expression of KrasG12D/+ or KrasG12V/+ alone does not show a similar phenotype; here the mice develop both adenoma and adenocarcinoma, but at very long latencies (>500 days) [44]. Thus, in mouse models, Apc mutation acts as an initiator, reducing latency and increasing tumour burden. This in itself is a problem, as the mice develop multiple tumours and thus may need to be euthanized due to burden before any tumours have had the opportunity to metastasize [79].

To overcome the issue of excessive tumour burden in mouse models, low-level recombination with
Cre-expressing viruses targeting the colon has been performed [48,80]. Using AdCre, Hung and colleagues developed a metastatic model of CRC, based around loss of Apc and Kras\textsubscript{G12D/+} mutation. One caveat of this model is the need for surgery, which may explain the surprising lack of uptake by the research community of what appears to be an excellent model.

Loss of TGFβ signalling is a common step during CRC progression. In the mouse, Apc mutation in combination with inactivation of various components of TGFβ signalling (Tgfbr2, Smad2, Smad3 or Smad4) generally leads to the production of invasive adenocarcinoma, although again not metastasis [81–84]. Smad3 loss in the Apc\textsuperscript{Min/+} model also altered tumour location, as more tumours arose in the distal colon [83]. One of the postulated mechanisms for how loss of TGFβ drives invasion (although it is required for processes such as EMT) is that mutations in the tumour lead to a protumourigenic (although it is required for processes such as EMT) is that mutations in the tumour lead to a protumourigenic tumour microenvironment. For example, the increased invasion observed in cis-Apc\textsuperscript{5716/+}Smad4\textsuperscript{-/-} mice was suggested to be mediated by recruitment of immature myeloid cells (iMCs) from the bone marrow, leading to secretion of matrix metalloproteinases (MMPs) at the invasion front of intestinal tumours [85].

The tumour-suppressor gene Tp53 is altered in 50–60% of human CRCs. Surprisingly, deletion of Tp53 in an outbred mouse background did not result in increased tumour progression in the Apc\textsuperscript{Min/+} model [86]. However, when analysed in a pure C57BL6/J background, Apc\textsuperscript{Min/+} Tp53\textsuperscript{-/-} compound mice revealed a tendency to higher tumour burden and the development of invasive tumours [87]. In human CRC, gain-of-function mutations of Tp53 are common, particularly Tp53\textsuperscript{R175H}. Expression of a single copy of the mouse version of this mutant, Tp53\textsuperscript{R172H/+}, with deletion of a single Apc allele, led to invasive tumour progression in all mice [88].

Collectively, it is clear that, when tested in mice, nearly all the common human mutations lead to increased tumour progression and development of adenocarcinoma, although alone these additional mutations do not provoke rapid tumourigenesis. One interesting hypothesis is that Apc mutation might make it harder for tumours to become metastatic in mice. This concept arose from work on two different targets of the Wnt pathway, Ephb2/3 and Tian1. EphrinB receptors (EphB) are direct Wnt target genes that control the architecture of the normal intestinal epithelium [89]. Interestingly, EPHB2, EPHB3 and EPHB4 are induced during early stages but down-regulated during CRC progression. Ephb3\textsuperscript{-/-} in the Apc\textsuperscript{Min/+} mice leads to conversion of 47% of tumours to adenocarcinoma, whilst ΔEphb2 deletion in the Apc\textsuperscript{Min/+} model reduces the number of tumours formed, but those tumours exhibit increased invasion [90]. Loss of Tian1, a pro-adhesive RAC–guanine nucleotide exchange factor (GEF), strongly suppresses tumourigenesis in the Apc\textsuperscript{Min/+} mice but resultant tumours are eventually invasive. Thus, it appears (at least in mice) that induction of the Wnt signalling programme favours benign tumour formation and thus additional mutations are required to drive further progression, which may in part overcome some of the pro-adhesive consequences of APC loss.

Modelling CRC metastasis with transplantation

Transplantation models are used to test pathways involved in invasion and metastasis that might be therapeutically targetable. These xenograft (human cell line) models result in desired characteristics, such as invasion and metastasis [91]. However, these characteristics are dependent on the route of inoculation. Subcutaneously injected tumour cells rarely, if ever, produce any metastases, but cells injected into the caecum, tail vein, spleen, portal vein or kidney capsule can metastasize to liver, lung and bones. Dependent on the site of injection, eg tail vein, many of the barriers that cancer cells face, which stop metastasis, such as extravasation or invasion through the basement membrane, may be lacking and it is important to remember these points. Experiments are performed in immune-compromised mice (widely used strains are nude or SCID mice) [92–97], and therefore lack a number of important tumour cell–host immune system interactions. Nevertheless, studies using human CRC cell lines have demonstrated the importance of the protumourigenic microenvironment. Orthotopically injected TGFβ-over-expressing HT29 and KM12L4a CRC cells activated IL-11 secretion from mouse cancer-associated fibroblasts, causing increased metastasis [98]. To overcome the problem of using immune-compromised mice, allografts of mouse CRC cell lines can be used. These have been very important for modelling immunotherapy strategies. For example, the cell lines CT26 and MCA38, which were generated from mouse colorectal tumours, have been injected orthotopically to the caecum and rectal wall of Balb/c and C57BL6/J mice, respectively, and have developed liver metastasis [93].

With the discovery of LGR5\textsuperscript{+} stem cells in the intestine, and following the isolation of these cells, Sato et al [99] developed ex vivo organoid cultures. These ‘mini-guts’ can be grown in a three-dimensional (3D) manner and they build tissue-like structures [99]. Outgrowth of wild-type spheres requires the presence of Paneth cells, which provide LGR5\textsuperscript{+} cells with niche factors [100]. These cultures therefore represent an excellent opportunity to model the mutations common in colon cancer. To manipulate gene expression in these organoids, a Cre recombinase-inducible retrovirus vector system has been developed [101]. Deletion of Apc in these organoids results in transformation, which is characterized by a morphological change to a more rounded spheroid shape and R-spondin-independent growth due to hyper-activated Wnt signalling [102]. Notably, these cells can be isolated from Villin–Cre\textsuperscript{ER}Apc\textsuperscript{B/A} crypts only 2 days after tamoxifen application to the mice. Additional mutation of Kras\textsuperscript{G12D/+} and Tp53\textsuperscript{R172H/+} or
deletion of Pten<sup>B/B</sup> confers the ability of these spheres to grow in nude mice [66,103–105]. The multi-hit theory proposed by Fearon and Vogelstein [2] was recapitulated in mouse organoids by simultaneous deletion of Apc, expression of Kras<sup>G12D/+</sup> and deletion of Tp53 and Smad4 (AKPS). These spheres have an invasive phenotype similar to that of human CRC [106].

Further validation of the sequential alteration of major pathways in CRC has now also been proved in organoids from normal human crypt stem cells, by using CRISPR/CAS9 technology [107]. The resulting AKPS cells show features of invasive carcinoma when subcutaneously injected into immunocompromised mice [9]. Another study described that Apc, Kras, Smad4, Tp53, PIK3CA<sup>E545K</sup> (AKSTP) mutant cells grow when engrafted under the kidney capsule of Nod–scid/IL2R<sup>γ</sup>-null mice. However, injection of these cells into the spleen gives rise only to micrometastases in the liver, whereas cells derived from human metastatic CRC form macrometastases. This work suggested that, in addition to the major driver mutations, further alterations are required for metastatic progression and for the outgrowth of CRC metastases in the liver [108].

Organoids may therefore help us decipher the consequences of the major mutations in CRC and be very useful in high-throughput screening for new therapies and potential therapeutic stratification. Already, much progress has occurred in the screening of tumour organoids from humans [109], providing promise for personalized/stratified therapy. It should be noted, however, that so far most of the screening has been done with organoid cultures in Matrigel<sup>®</sup>, and it will be important to see how microenvironmental changes and the culture of spheres might alter the response of these drugs; we have shown that basic properties, such as the ratio of E-cadherin/β-catenin, are very different in the in vivo setting versus cell culture [110]. These new organoid models should lead to both the reduction and replacement of animal experiments. Given the need to test therapies in a 3D environment with an intact tumour stroma, there is still a very important role for autochthonous models, but hopefully experiments performed in organoids will predict in vivo responses better than other model systems.

**GEMMs of metastatic intestinal cancer**

One of the major goals of utilizing mouse models of cancer is to recapitulate the human disease in order to produce models to test treatments. Thus, to predict response in this setting, we need models that metastasize and these models are still lacking. Thus far, most of our more successful models of metastasis are still of long latency and low penetrance. Also, most of these models do not carry mutation of APC. In this section we will describe these models.

In addition to the classical model of CRC progression, alternative routes to CRC have been described [6]. One alternative route is the serrated route, which is characterized by hyperplastic lesions and a saw-toothed (serrated) histology of the intestinal epithelium [111]. Molecular differences between the classical and serrated route also exist. The serrated route is characterized by initial BRAF or KRAS mutations and no APC mutations [112]. In a mouse model of serrated CRC, the expression of oncogenic Braf<sup>L597S-V637E/+</sup> from its endogenous promoter led to the full progression of serrated hyperplasia to adenoma and finally to metastatic carcinoma. However, latency was long and the percentage of metastasis was low with Braf<sup>L597S-V637E/+</sup> alone (one of five mice). A possible increase in metastasis was detected when mutant Tp53<sup>R172H/+</sup> (three of 12 mice) or p16<sup>B/B</sup> (three of 12 mice) were also mutated in addition to Braf mutation, but latency and penetrance were still low [113]. A further model of serrated tumourigenesis that progresses to adenocarcinoma was driven by mutation of Kras<sup>G12V/+</sup> and Pten<sup>B/B</sup> deletion; here, 41% of mice developed metastasis, with over half developing in the liver [114]. Another model that has shown metastasis is Kras<sup>G12D/+</sup> mice combined with deletion of Tgfbr2; here, CRC cells spread to local lymph nodes and the lung in 15% of the mice. This dysplastic progression was triggered by hyper-activated EGFR signalling [115].

Lung metastasis was detected in 62% of mice with concomitant Kras<sup>G12D/+</sup> activation and Ink4a/Arf<sup>−/−</sup> deletion; primary invasive tumours showed serrated morphology and p16-dependent depression of senescence [116]. It is interesting to note that all these models have in common a long latency and a lack of Apc mutation. However, in all models, high levels of Wnt signalling were observed in the adenocarcinoma and metastases that arose, suggesting that Wnt activation may progress these lesions from serrated lesions into ‘bona fide’ adenocarcinomas. The relevance of these serrated models has recently come to the fore, given that CRCs which have the poorest prognosis often have a ‘serrated’ signature [117].

Notch signalling is a key regulator of intestinal epithelial cell fate during normal homeostasis and contributes to tumour development [118]. Genetic alterations in the Notch pathway leading to human CRC have not been reported. However, FBXW7 is altered in 20% of human CRCs and can control Notch receptor stability [119]. The function of Notch signalling in intestinal mouse models is controversial, as over-expression of the intracellular active domain of the Notch-receptor 1 (Nicd1<sup>LSL–GFP</sup>) in combination with the Apc<sup>Mnt+</sup> mutation generates higher numbers of adenomas which were higher-differentiated compared to the control [120]. However it has recently been shown that aberrant expression of Nicd1 in combination with Tp53 deletion in the mouse intestine generates adenocarcinomas that exhibit markers of EMT. Analysis of these mice revealed that 23% had lymph node infiltration and 10% showed spread of tumour cells to the liver [121]. Lymph node infiltration with an EMT of the primary tumour has also been reported when Tp57 was deleted in IEC and mice where challenged with AOM [122]. It will be therefore of interest to discover whether any of these models can
produce metastasis with a higher penetrance and faster latency when further oncogenic/tumour suppressor mutations are added.

Other species

During recent years, other animal models of CRC have been developed in both rats and pig. Both, especially the pig, can recapitulate human physiology and pharmacology in a much better way than mice. In rats, two models of CRC were developed by administration of ENU, the same mutagen used for generating the Apc\textsuperscript{Min/+} mouse [123,124]. The most appropriate of these is the Apc\textsuperscript{Pin/+} rat, which harbours a mutation in Apc which converts lysine → Stop at codon 1137 [123]. These rats exhibit strikingly similar pathology to human CRC, with the development of tumours with intramuscular invasion [125,126]. The porcine model of FAP was created by generating porcine ES cells carrying an Apc\textsuperscript{1311} mutation [127]. Germline heterozygous pigs were developed that went on to develop multiple polyps by age of 1 year (both low- and high-grade dysplasia) and so act as an excellent model of FAP. Taken together, these new models open new avenues to model early-stage human CRC, but still lack metastasis.

Conclusion and future work

It is 25 years since the publication of reports of the Apc\textsuperscript{Min/+} mouse and this model has been extensively used to characterize the mechanism, modifiers and potential therapeutic strategies for early-stage intestinal tumourigenesis [128]. Development of models that more closely mimic late-stage disease for routine use by the community have lagged well behind, so there is not a routine GEMM for CRC that has a short latency and high penetrance. The recent excitement over new subtypes of CRC and potential stratification of patients by mutation and/or subtype makes the need for model systems more important than ever. Moreover, as immunotherapy trials become more and more the norm in cancer research, the need for immunocompetent autochthonous models to test rational combinations is vital. The advent of organoids over the past 10 years from both mouse and human normal intestine and cancer offers excellent new model systems. Transplantation of these are currently non-orthotopic but in the future orthotopic injection may provide new models of metastatic CRC. GEMMs will remain vital to understand how the common co-existing mutations cooperate in a natural environment. Current challenges are to assess how stroma and microbiota affect drug response, and these will need to be performed in situ. While we have not succeeded so far in the development of metastatic CRC models, many fundamental discoveries have been made about stem cells, homeostasis and transformation, so the community has failed very successfully! Our future aims must be to better model, understand and treat the later stages of CRC.

Acknowledgements

We apologise to those whose work could not be cited due to space limitation. RJ is a Marie Sklodowska-Curie Actions research fellow. OJS is funded by Cancer Research UK (Core Grant No. A12481) and an ERC Consolidator Award (ColonCan, Grant No. 311301) and is supported by Cancer Research UK (Grant Nos A18076 and A17196).

Author contributions

OJS and RJ wrote the manuscript.

References

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clinic 2011; 61: 69–90.
2. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759–767.
3. Sottoriva A, Kang H, Ma Z, et al. A Big Bang model of human colorectal tumor growth. Nat Genet 2015; 47: 209–216.
4. Morin PJ, Sparks AB, Korinek V, et al. Activation of β-catenin–Tcf signaling in colon cancer by mutations in β-catenin or APC. Science 1997; 273: 1787–1790.
5. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. Cell 2000; 103: 311–320.
6. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol 2011; 6: 479–507.
7. Hedrick L, Cho KR, Fearon ER, et al. The DCC gene product in cellular differentiation and colorectal tumorigenesis. Genes Dev 1994; 8: 1174–1183.
8. Fearon ER, Cho KR, Nigro JM, et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 1990; 247: 49–56.
9. Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. Nature 2015; 521: 43–47.
10. Rajagopalan H, Nowak MA, Vogelstein B, et al. The significance of unstable chromosomes in colorectal cancer. Nat Rev Cancer 2003; 3: 695–701.
11. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. Gastroenterology 2010; 138: 2059–2072.
12. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. Science 2013; 339: 1546–1558.
13. Cancer Genome Atlas. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487: 330–337.
14. March HN, Rust AG, Wright NA, et al. Insertional mutagenesis identifies multiple networks of cooperating genes driving intestinal tumorigenesis. Nat Genet 2011; 43: 1202–1209.
15. Markowitz S, Wang J, Myeroff L, et al. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. Science 1995; 268: 1336–1338.
16. Scherer SJ, Avdievich E, Edelmann W. Functional consequences of DNA mismatch repair missense mutations in murine models and their impact on cancer predisposition. Biochem Soc Trans 2005; 33: 689–693.
17. Wei K, Kucherlapati R, Edelmann W. Mouse models for human DNA mismatch–repair gene defects. Trends Mol Med 2002; 8: 346–353.
18. Wojciechowicz K, Cantelli E, Van Gerwen B, et al. Temozolomide increases the number of mismatch repair-deficient intestinal crypts
and accelerates tumorigenesis in a mouse model of Lynch syndrome. Gastroenterology 2014; 147: 1064–1072, e1065.

Kelderman S, Schumacher TN, Kvisbjerg P. Mismatch repair-deficient cancers are targets for anti-PD-1 therapy. Cancer Cell 2015; 28: 11–13.

Wang K, Karin M. Tumor-elicited inflammation and colorectal cancer. Adv Cancer Res 2015; 128: 173–196.

Quante M, Varga J, Wang TC, et al. The gastrointestinal tumor microenvironment. Gastroenterology 2013; 145: 63–78.

Groden J, Thliveris A, Samowitz W, et al. The genetics of hereditary colon cancer. New England J Med 2000; 342: 891–899.

Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. NEnglJMed 2003; 348: 891–899.

Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003; 348: 883–890.

Shibata H, Toyama K, Shioya H, et al. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science 1997; 278: 120–123.

Sansom OJ, Rees KR, Hayes AJ, et al. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. Genes Dev 2004; 18: 1385–1390.

Andreu P, Colnot S, Godard C, et al. Crypt-restricted proliferation and commitment to the Paneth cell lineage following Apc loss in the mouse intestine. Development 2005; 132: 1443–1451.

Akyol A, Hinoi T, Feng Y, et al. Generating somatic mosaicism with a Cre recombinase–microsatellite sequence transgene. Nat Methods 2008; 5: 231–233.

The gastrointestinal tumor microenvironment. Gastroenterology 2013; 145: 63–78.

The secretory phospholipase A2 gene is a candidate for the modifier of APCMin-induced intestinal neoplasia. Cell 1993; 75: 631–639.

McCart AE, Vickaryous NK, Silver A. Apc mice: models, modifiers and mutants. Pathol Res Pract 2008; 204: 479–490.

Young M, Ordonez L, Clarke AR. What are the best routes to effectively model human colorectal cancer? Mol Oncol 2013; 7: 178–189.

Gould KA, Dietrich WF, Borenstein N, et al. Mm01 is a semi-dominant modifier of intestinal adenoma size and multiplicity in Min+ mice. Genetics 1996; 144: 1769–1776.

Moser AR, Dove WF, Roth KA, et al. The Min (multiple intestinal neoplasia) mutation: its effect on gut epithelial cell differentiation and interaction with a modifier factor. J Cell Biol 1992; 116: 1517–1526.

Li Y, Kandu P, Seow SW, et al. Gut microbiota accelerate tumor growth via e-cad and STAT3 phosphorylation in APCMin+ mice. Carcinogenesis 2012; 33: 1231–1238.

Mai V, Colbert LH, Berriegan D, et al. Calorie restriction and diet composition modulate spontaneous intestinal tumorigenesis in ApcMin mice through different mechanisms. Cancer Res 2003; 63: 1752–1755.

Pollard P, Deheragoda M, Segritis S, et al. The Apc 1322 T mouse develops severe polyposis associated with submaximal nuclear β-catenin expression. Gastroenterology 2009; 136: 2204–2213, e2201–2213.

Cheung AF, Carter AM, Kostova KK, et al. Complete deletion of Apc results in severe polyposis in mice. Oncogene 2010; 29: 1857–1864.

Lewis A, Segritis S, Deheragoda M, et al. Severe polyposis in Apc (1322 T) mice is associated with submaximal Wnt signalling and increased expression of the stem cell marker Lgr5. Gut 2010; 59: 1680–1686.

Fodde R, Edelmann W, Yang K, et al. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. Proc Natl Acad Sci USA 1994; 91: 8969–8973.

Nagy A. Cre recombinase: the universal reagent for genome tailoring. Genesis 2000; 26: 99–109.

Sansom OJ, Meniel V, Wilkins JA, et al. Loss of Apc allows phenotypic manifestation of the transforming properties of an endogenous K-ras oncogene in vivo. Proc Natl Acad Sci USA 2006; 103: 14122–14127.

Jackson EL, Willis N, Mercer K, et al. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001; 15: 3243–3248.

Hayashi S, McMahon AP. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. Dev Biol 2002; 244: 305–318.

Akyol A, Hinoi T, Feng Y, et al. Generating somatic mosaicism with a Cre recombinase–microsatellite sequence transgene. Nat Methods 2008; 5: 231–233.

Shibata H, Toyama K, Shioya H, et al. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. Science 1997; 278: 120–123.

Sansom OJ, Rees KR, Hayes AJ, et al. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. Genes Dev 2004; 18: 1385–1390.

Andreu P, Colnot S, Godard C, et al. Crypt-restricted proliferation and commitment to the Paneth cell lineage following Apc loss in the mouse intestine. Development 2005; 132: 1443–1451.

Sansom OJ, Meniel VS, Muncan V, et al. Muc deletion rescues Apc deficiency in the small intestine. Nature 2007; 446: 676–679.

Athineos D, Sansom OJ. Muc heterozygosity attenuates the phenotypes of APC deficiency in the small intestine. Oncogene 2010; 29: 2585–2590.

Ashton GH, Morton JP, Myant K, et al. Focal adhesion kinase is required for intestinal regeneration and tumorigenesis downstream of Wnt/β-catenin signaling. Dev Cell 2010; 19: 259–269.

Myant KB, Cammareri P, McGhee EJ, et al. ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. Cell Stem Cell 2013; 12: 761–773.

Cole AM, Myant K, Reed KR, et al. Cyclin D2-cyclin-dependent kinase 4/6 is required for efficient proliferation and tumorigenesis following Apc loss. Cancer Res 2010; 70: 8149–8158.

Feng Y, Sentani K, Wiese A, et al. SOX9 induction, ectopic Paneth cells, and mitotic spindle axis defects in mouse colon adenomatous epithelium arising from conditional biallelic Apc inactivation. Am J Pathol 2013; 183: 493–503.

Malaterre J, Carpinelli M, Ernst M, et al. c-Myb is required for progenitor cell homeostasis in colonic crypts. Proc Natl Acad Sci USA 2007; 104: 3829–3834.

Hinoi T, Akyol A, Theisen BK, et al. Mouse model of colonic adenoma–cancer progression based on somatic Apc inactivation. Cancer Res 2007; 67: 9721–9730.

Robanus-Maandag EC, Koelink PJ, Breukel C, et al. A new conditional Apc-mutant mouse model for colorectal cancer. Carcinogenesis 2010; 31: 946–952.
60. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 2007; 449: 1003–1007.

61. de Lau W, Barker N, Low TY, et al. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 2011; 476: 291–297.

62. Barker N, Ridgway RA, van Es JH, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 2009; 457: 608–611.

63. Powell AE, Vlacich G, Zhao ZY, et al. Inducible loss of one Apc allele in Lrig1-expressing progenitor cells results in multiple distal colonic tumors with features of familial adenomatous polyposis. Am J Physiol Gastrointest Liver Physiol 2014; 307: G16–23.

64. Sangiorgi E, Capecchi MR. Bmi1 is expressed in vivo in intestinal stem cells. Nat Genet 2008; 40: 915–920.

65. Zhu L, Gibson P, Currie DS, et al. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature 2009; 457: 603–607.

66. Schwittalla S, Fingerle AA, Cammareri P, et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem cell-like properties. Cell 2013; 152: 25–38.

67. Westphalen CB, Asfaha S, Hayakawa Y, et al. Long-lived intestinal tuft cells serve as colon cancer-initiating cells. J Clin Invest 2014; 124: 1283–1295.

68. Shih IM, Wang TL, Traverso G, et al. Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci USA 2001; 98: 2640–2645.

69. Janssen KP, Alberici P, Fsihi H, et al. APC139 mutations are synergistic in the development of intestinal cancers. Nature 1998; 92: 1762–1772.

70. Halberg RB, Katzung DS, Hoff PD, et al. Tumorigenesis in the multiple intestinal neoplasia mouse: redundancy of negative regulators and specificity of modifiers. Proc Natl Acad Sci USA 2000; 97: 3461–3466.

71. Muller PA, Caswell PT, Doyle B, et al. Mutant p53 drives invasion by promoting integrin recycling. Cell 2009; 139: 1327–1341.

72. Battle E, Henderson JT, Begthel H, et al. β-Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB4 and EphrinB2. Cell 2002; 111: 251–263.

73. Batte E, Bacani J, Begthel H, et al. EphB receptor activity suppresses colorectal cancer progression. Nature 2005; 435: 1126–1130.

74. Fieler D. Orthotopic implantation of human colon carcinomas into nude mice provides a valuable model for the biology and therapy of metastasis. Cancer Metast Rev 1991; 10: 229–243.

75. Sun FX, Sasson AR, Jiang P, et al. An ultra-metastatic model of human colon cancer in nude mice. Clin Exp Metast 1999; 17: 41–48.

76. Kashtan H, Rahab M, Mullen JB, et al. Intra-rectal injection of tumour cells: a novel animal model of rectal cancer. Surg Oncol 1992; 1: 251–256.

77. Cespedes MV, Espina C, Garcia-Cabezas MA, et al. Orthotopic microinjection of human colon cancer cells in nude mice induces tumor foci in all clinically relevant metastatic sites. Am J Pathol 2007; 170: 1077–1085.

78. Giavazzi R, Jessup JM, Campbell DE, et al. Experimental nude mouse model of colorectal cancer liver metastases. J Natl Cancer Inst 1986; 77: 1303–1308.

79. Bankert RB, Egilmez NK, Hess SD. Human–SCID mouse chimeric models for the evaluation of anti-cancer therapies. Trends Immunol 2001; 22: 386–393.

80. Ogata Y, Hara Y, Akagi Y, et al. Metastatic model of human colon cancer constructed using orthotopic implantation in nude mice. Kurume Med J 1998; 45: 121–125.

81. Colon A, Espinet E, Palomo-Ponce S, et al. Dependency of colorectal cancer on a TGFβ-driven program in stromal cells for metastasis initiation. Cancer Cell 2012; 22: 571–584.

82. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt–villus structures in vitro without a mesenchymal niche. Nature 2009; 459: 262–265.

83. Calon A, Van Es JH, Snippert HJ, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 2011; 469: 415–418.

84. Koo BK, Stange DE, Sato T, et al. Controlled gene expression in primary Lgr5 organoid cultures. Nat Methods 2012; 9: 81–83.

85. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. Gastroenterology 2011; 141: 1762–1772.

86. Valeri N, Bracconeri C, Gasparini P, et al. MicroRNA-135b promotes cancer progression by acting as a downstream effector of oncogenic pathways in colon cancer. Cancer Cell 2014; 25: 469–483.
Mouse models of advanced CRC

104. van Es JH, Clevers H. Generation and analysis of mouse intestinal tumors and organoids harboring APC and K-Ras mutations. Methods Mol Biol 2015; 1267: 125–144.

105. Huel DJ, Cammareri P, Ridgway RA, et al. Methods to assess Myc function in intestinal homeostasis, regeneration, and tumorigenesis. Methods Mol Biol 2013; 1012: 237–248.

106. Li X, Nadauld L, Ootani A, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. Nat Med 2014; 20: 769–777.

107. Rn FA, Hsu PD, Lin CY, et al. Double nicking by RNA-guided CRISPR-Cas9 for enhanced genome editing specificity. Cell 2013; 154: 1380–1389.

108. Matano M, Date S, Shimokawa M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nat Med 2015; 21: 256–262.

109. van de Wetering M, Francis HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015; 161: 933–945.

110. van Es JH, Ridgway RA, Radulescu S, et al. E-cadherin can limit the transforming properties of activating β-catenin mutations. EMBO J 2015; 34: 2321–2333.

111. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. J Pathol 2007; 50: 113–130.

112. Rad R, Cadinanos J, Rad L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. Cancer Cell 2013; 24; 15–29.

113. Davies EI, Marsh Durban V, Meniel V, et al. PTEN loss and KRAS activation leads to the formation of serrated adenomas and metastatic carcinoma in the mouse intestine. J Pathol 2014; 233: 27–38.

114. Trobridge P, Knowlaugh S, Washington MK, et al. TGFβ receptor inactivation and mutant Kras induce intestinal neoplasms in mice via a β-catenin-independent pathway. Gastroenterology 2009; 136: 1680–1688, e1687.

115. Bennecke M, Kriegl L, Bajouj M, et al. Ink4a/Arf and oncogene-induced senescence prevent tumor progression during alternative colorectal tumorigenesis. Cancer Cell 2010; 18: 135–146.

116. De Sousa EMF, Wang X, Jansen M, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 2013; 19: 614–618.

117. Radtke F, Clevers H, Riccio O. From gut homeostasis to cancer. Curr Mol Med 2006; 6: 275–289.

118. Tan Y, Sangfelt O, Spruck C. The Fbxw7/Hsd1 tumor suppressor in human cancer. Cancer Lett 2008; 271: 1–12.

119. Kim HA, Koo BK, Cho JH, et al. Notch1 counteracts WNT/β-catenin signaling through chromatin modification in colorectal cancer. J Clin Invest 2012; 122: 3248–3259.

120. Chanrion M, Kuperstein I, Barriere C, et al. Concomitant Notch activation and p53 deletion trigger epithelial-to-mesenchymal transition and metastasis in mouse gut. Nat Commun 2014; 5: 5005.

121. Schwitalla S, Ziegler PK, Horst D, et al. Loss of p53 in enterocytes generates an inflammatory microenvironment enabling invasion and lymph node metastasis of carcinogen-induced colorectal tumors. Cancer cell 2013; 23: 93–106.

122. van Boxtel R, Cuppen E. Generation of genetically modified rodents using random ENU mutagenesis. Methods Mol Biol 2011; 693: 295–308.

123. Mashimo T, Yanagihara K, Tokuda S, et al. An ENU-induced mutant archive for gene targeting in rats. Nat Genet 2008; 40: 514–515.

124. Washington MK, Powell AE, Sullivan R, et al. Pathology of rodent models of intestinal cancer: progress report and recommendations. Gastroenterology 2013; 144: 705–717.

125. Irving AA, Yoshimi K, Hart ML, et al. The utility of Apc-mutant rats in modeling human colon cancer. Dis Model Mecham 2014; 7: 1215–1225.

126. Flisikowska T, Merkl C, Landmann M, et al. A porcine model of familial adenomatous polyposis. Gastroenterology 2012; 143; 1173–1175, e1171–1177.

127. Faller WJ, Jackson TJ, Knight JR, et al. mTORC1-mediated translational elongation limits intestinal tumour initiation and growth. Nature 2015; 517: 497–500.

50 Years ago in the Journal of Pathology...

Urogenital lesions in laboratory mice
A. A. Tuffery

To view these articles, and more, please visit: www.thejournalofpathology.com

Click ‘ALL ISSUES (1892 - 2015)’, to read articles going right back to Volume 1, Issue 1.