


Shortcuts to intestinal carcinogenesis by genetic engineering in organoids

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Inactivation of the *Adenomatous polyposis coli* (APC) gene is an initiating and the most relevant event in most sporadic cases of colorectal cancer, providing a rationale for using *Apc*-mutant mice as the disease model. Whereas carcinogenesis has been observed only at the organism level, the recent development of the organoid culture technique has enabled long-term propagation of intestinal stem cells in a physiological setting, raising the possibility that organoids could serve as an alternative platform for modeling colon carcinogenesis. Indeed, it is demonstrated in the present study that lentivirus-based RNAi-mediated knockdown of *Apc* in intestinal organoids gave rise to subcutaneous tumors upon inoculation in immunodeficient mice. Reconstitution of common genetic aberrations in organoids resulted in development of various lesions, ranging from aberrant crypt foci to full-blown cancer, recapitulating multi-step colorectal tumorigenesis. Due to its simplicity and utility, similar organoid-based approaches have been applied to both murine and human cells in many investigations, to gain mechanistic insight into tumorigenesis, to validate putative tumor suppressor genes or oncogenes, and to establish preclinical models for drug discovery. In this review article, we provide a multifaceted overview of these types of approaches that will likely accelerate and advance research on colon cancer.

KEYWORDS

Apc, colon cancer, lentivirus, model, organoid

1 | INTRODUCTION

Carcinoma arises from epithelial cells through accumulation of genetic aberrations^{1,2} and the tissue-specific microenvironment could further promote this process. More than 3 decades ago, rats served as a major animal model of human carcinogenesis, particularly for those types of carcinogenesis induced by chemical carcinogens.^{3,4} For example, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a food-borne carcinogen in well-cooked meat, induced tumor development in colon, prostate and mammary glands in rats,⁵ which

was significantly promoted by a high-fat diet,⁶ while it preferentially induced lymphoma in mice.⁷ Considering the sharp increase in deaths from these types of cancers, coinciding with the Westernization of diet in Japan,⁸ PhIP-induced carcinogenesis models in rats, indeed, phenocopied many aspects of the pathogenesis of the human disease.

Ever since genetically engineered mice (GEM) became available as a result of gene-targeting technology,^{9,10} mice have been the gold standard in modeling cancer. GEM with conditional alleles could also be generated by using the Cre-loxP system to achieve recombination in a tempo-spatially controlled manner,¹¹ paving the way

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for organ-specific or adult-onset carcinogenesis studies.¹² Despite these benefits, critical drawbacks of GEM might include that its generation could take a long time and be laborious. This is particularly true if conditional gene targeting and multiple intercrossing are required. In addition, modifier genes could become problematic when intercrossing between different strains. Indeed, cooperation between *Apc* inactivation and *Trp53* loss towards intestinal tumorigenesis became evident only in the C57BL/6J background after backcrossing for many generations.^{13,14}

Matrigel-based organoid culture has recently emerged as a technique that essentially recapitulates tissue homeostasis in vitro exclusively with epithelial cells.¹⁵ It is likely that laminin, abundantly contained in Matrigel,¹⁶ and defined factors can reconstitute the intestinal stem cell niche. Both self-renewal and differentiation of stem cells are maintained, thereby enabling infinite proliferation of stem cells without forced immortalization or transformation. By using this technique, we demonstrated that transformation of murine normal intestinal cells was feasible, through in vitro gene transduction and inoculation in nude mice.¹⁷ Importantly, induced carcinogenesis was highly concordant with earlier studies using GEM-based in vivo models.¹⁸ Due to its simple and rapid nature, genetic engineering of organoids might be established as a next generation model of carcinogenesis. In this article, we illustrate the technical basics of this new approach, and review studies by others with similar techniques.

2 | CONVENTIONAL MOUSE MODELS FOR INTESTINAL TUMOR DEVELOPMENT

In human colorectal cancer (CRC), mutations in the *Adenomatous polyposis coli* (*APC*) gene and the *CTNNB1* gene encoding β -catenin are found in > 80% and about 10%, respectively.¹⁹ Both mutations result in nuclear accumulation of β -catenin, which cooperates with TCF4 to establish constitutive transcriptional activation of the Wnt pathway.²⁰ Reflecting its critical role in tumor initiation, mouse models for intestinal carcinogenesis are based on the genetic aberrations in either gene.^{21,22} For any candidate gene related to CRC, GEM are usually generated first and intercrossed with these models to evaluate the impact on tumor progression^{23,24} by examining the changes in the incidence, size, multiplicity and histology of the tumors (Figure 1A).

The *APC* gene is typically inactivated by truncating mutations.²⁵ *Apc*^{Min/+} mice carrying the truncated allele were generated through *N*-ethyl-*N*-nitrosourea (ENU)-based mutagenesis.²⁶ While homozygous ablation of *Apc* results in embryonic lethality, heterozygously mutant mice spontaneously develop numerous adenomatous polyps after the second hit in *Apc*,^{27,28} resembling CRC-prone hereditary syndrome familial adenomatous polyposis (FAP), except that tumors were predominantly observed in the small intestine.^{29,30} Mice with a conditional allele of mutant *Apc* (eg, *Apc*^{fllox/+}) intercrossed with *villin*-Cre mice also developed polyps preferentially in the small intestine.³¹ These observations

strongly suggest that small intestinal cells are more susceptible to transformation by *Apc* inactivation than colonic cells in mice. To develop cancer in the colon, *Apc*^{fllox/+} mice were intercrossed with transgenic mice that preferentially expressed Cre in the large intestine, such as *Cdx2*-Cre³¹ or *CAC*-Cre,³² or an adenovirus encoding Cre (adeno-Cre) was directly infused into the rectum of *Apc*^{fllox/fllox}.³³ Colon carcinogenesis can be alternatively initiated by treatment with the carcinogen azoxymethane (AOM),³⁴ which efficiently introduces an activating mutation in the *Ctnnb1* gene in the murine colon,³⁵ and promoted by dextran sulphate sodium (DSS) to induce colitis.³⁶

3 | AN ORGANOID-BASED CARCINOGENESIS MODEL FOR MURINE INTESTINE

The NIH3T3-based transformation assay in nude mice (Figure 1B) has contributed to the validation of oncogenic potential of many genes.^{37,38} Given the development of the organoid culture for murine small intestinal cells,¹⁵ we reasoned that similar approaches with epithelial cells might become feasible. As a proof-of-concept experiment, knockdown of *Apc* and other tumor suppressor genes were achieved by lentiviral delivery of corresponding short-hairpin RNA (shRNA), either alone or in combination, into primary intestinal organoids from wildtype mice with the C57BL/6J background (Figure 1C). Transduced organoids were inoculated in the subcutis of nude mice and monitored for tumor development. We here illustrate the key technical features of this model.¹⁷

3.1 | Matrigel-bilayer organoid culture for primary culture, passage and infection

Intestinal crypts or singly dissociated cells are usually resuspended in liquid Matrigel to form a dome-like structure on a culture dish¹⁵ (Figure 2). In an effort to establish robust lentiviral infection in intestinal organoids, we found that single cells or organoids in Matrigel were resistant to lentiviral infection. However, they immediately died in the absence of Matrigel. To circumvent this issue, we plated the single cells on Matrigel and co-incubated overnight with viral particles. After removing the virus and the floating dead cells the next day, the cells attaching to the Matrigel were covered with Matrigel. This simple procedure, referred to as Matrigel bilayer organoid culture (MBOC), achieved significantly high infection efficiency (approximately 90%) and robust propagation of transduced organoids³⁹ (Figure 2). Consequently, we did not use the spin infection method, although widely used for hematological cells,^{40,41} to avoid the risk of damaging naive single epithelial cells with several hours of centrifugation. We eventually adopted MBOC for the routine passage and primary culture as well, because it efficiently eliminated dead or differentiated cells that could digest Matrigel or exert toxic effects on viable cells, and robustly captured highly proliferative

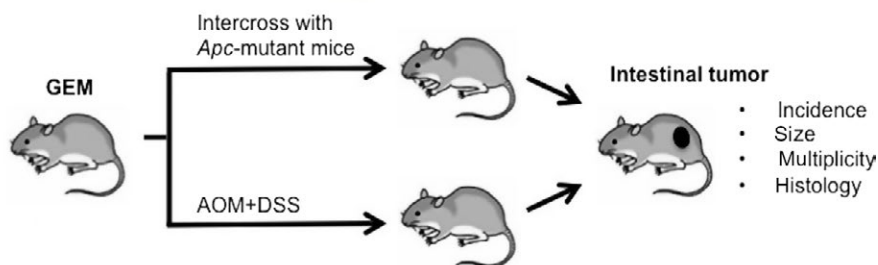
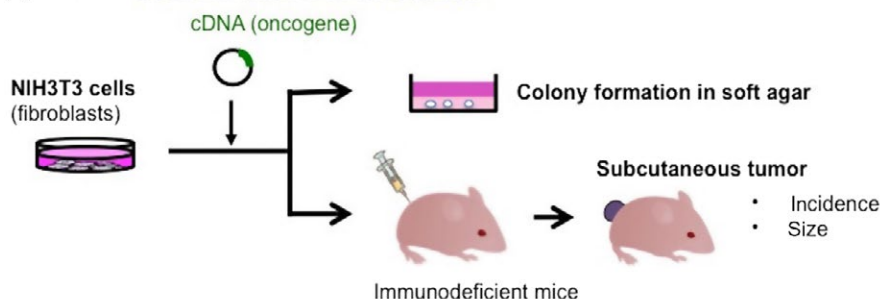
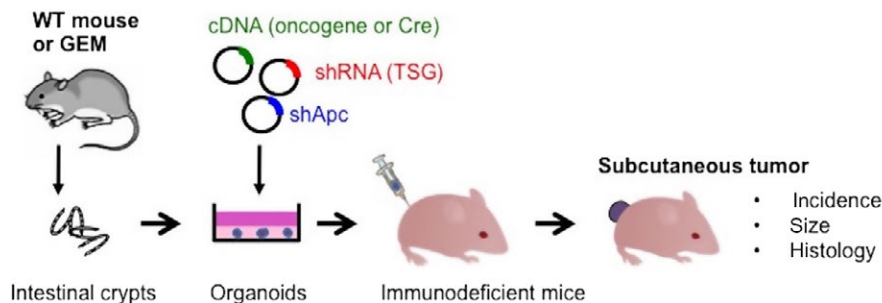
(A) In vivo and in situ carcinogenesis**(B) In vitro transformation of fibroblasts****(C) Ex vivo and heterotopic carcinogenesis**

FIGURE 1 Models to validate tumorigenic potential of a candidate gene. A, In vivo mouse models for colon carcinogenesis. Genetically engineered mice (GEM) for a candidate gene is generated and intercrossed with *Apc*-mutant mice or treated with azoxymethane (AOM)/dextran sulphate sodium (DSS) to monitor impact of the gene on tumor development or further progression. Representative readouts are shown. B, Cell-based transformation assay. Candidate oncogenes are introduced into immortalized murine fibroblasts NIH3T3, followed by culture in soft agar or inoculation in the subcutis of nude mice. C, An ex vivo organoid-based model. Primary organoid culture of intestinal cells is followed by lentiviral gene transduction. Organoids transduced with cDNA and/or shRNA are inoculated in immunodeficient mice to monitor tumor development

stem-like cell populations on Matrigel, without conducting cell sorting by stem cell markers.

3.2 | Re-defining organoid-derived tumors in the subcutis of nude mice

As readouts for the tumorigenicity in mice models, it is common to take the incidence, multiplicity, size and histology of the tumors into account.⁴² To evaluate the results from organoid-derived carcinogenesis models, however, it was necessary to newly determine criteria for the diagnosis of “tumors.” Transduced intestinal organoids mixed with Matrigel formed subcutaneous nodules in nude mice, which fell into 4 categories based on their macroscopic and microscopic features. The former 2 were regarded as non-tumors and the latter 2 as tumors (Figure 3).¹⁷

3.2.1 | Matrigel plug

Unless *Apc* is silenced in inoculated organoids, no nodule can be detected, yet flat white semi-transparent materials occasionally

remained in the injection sites. Microscopically, no epithelial cells were observed, while fibroblasts, immune cells or calcified remains of organoids could be detected. These materials without viable epithelial glands are defined as Matrigel plugs.

3.2.2 | Non-tumorous nodule

Matrigel plug-like nodules could contain a tiny population of epithelial glands. Histologically, a few non-dysplastic tubular glands are lined up in a monolayer. Despite the presence of epithelial glands in the subcutis, these lesions were classified as non-tumorous nodules, as bona fide tumors would have potentially outgrown Matrigel. No viable organoids were, in fact, recovered from these nodules, denying the presence of tumor-initiating cells. It is possible that organoids acquired a growth advantage as a result of random genomic integration of lentiviral vectors.⁴³

Organoids expressing *Kras*^{G12D} can be generated by delivery of Cre into organoids from conditional knock-in mice heterozygous for the LSL-*Kras*^{G12D} allele.⁴⁴ Upon inoculation, a few enlarged glands could be transiently formed in non-tumorous nodules, but eventually

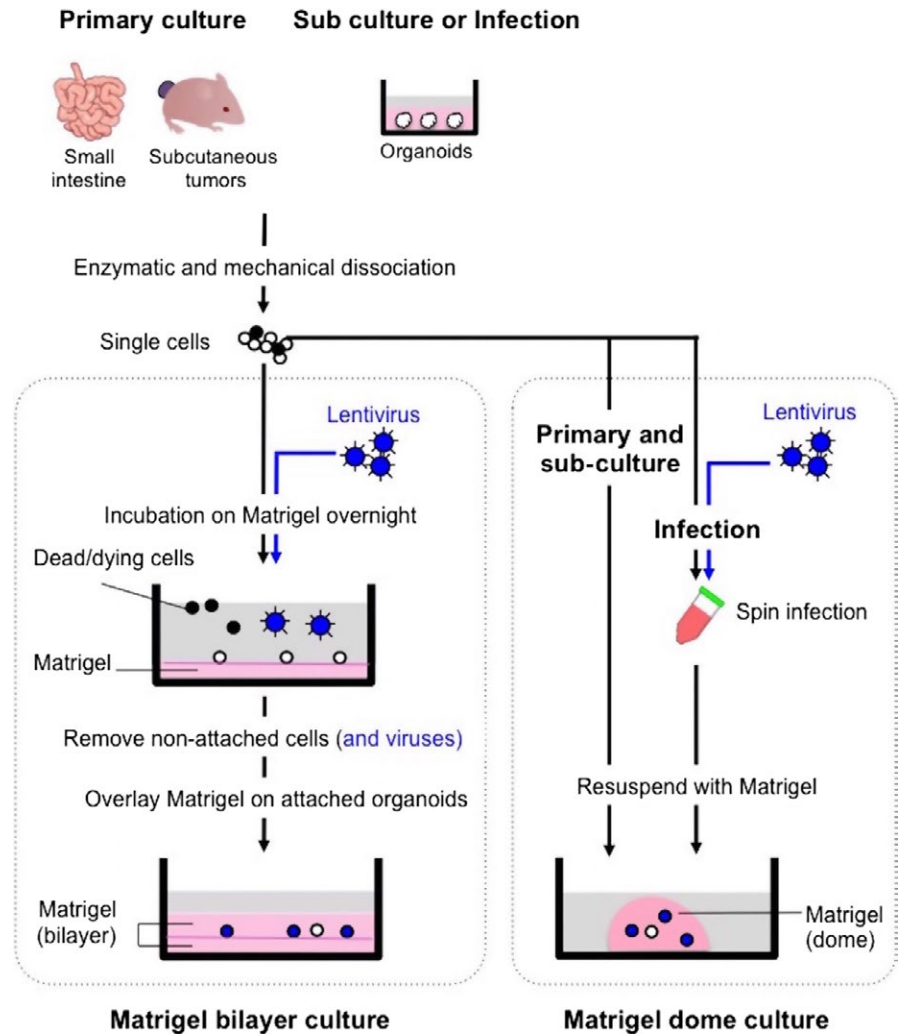


FIGURE 2 Matrigel bilayer culture for propagation and infection of organoids. Epithelial cells from normal small intestine and subcutaneous tumors can be propagated as organoids. Left panel: Matrigel bilayer culture. Singly dissociated cells are plated on Matrigel, and the cells attached on the lower layer of Matrigel are further covered with Matrigel the next day. By adopting Matrigel bilayer organoid culture, dead cells and tissue-derived debris can be promptly eliminated from the culture, facilitating both primary- and sub-culture and enabling efficient lentiviral infection. Open circles and closed circles depict viable single cells and dead cells, respectively. Lentivirally transduced cells are labeled in blue. Right panel: Matrigel dome culture. Cells are directly resuspended in Matrigel and poured on a dish to form a dome-like structure. Spin infection is conducted in a tube

disappeared to form Matrigel plugs,¹⁷ consistent with the low tumorigenic potential of *Kras*^{G12D} alone in vivo.^{45,46} Similar histology and disappearance have been observed in chemically-induced aberrant crypt foci (ACF), putative early lesions in colon carcinogenesis.⁴⁷ Whereas dysplastic ACF represent a pre-neoplastic lesion,^{48,49} hyperplastic ACF are associated with *Kras* mutation but do not progress into tumors.^{50,51} Thus, hyperplastic ACF might also be present, at least temporarily, in non-tumorous nodules.

3.2.3 | Solid tumor

A solid tumor is defined as a palpable round-shaped nodule. It typically has a similar color to white muscle, and consists of tubular glands accompanied by stromal infiltration. Co-injected Matrigel is no longer observed, indicating that the tumor outgrew and substituted Matrigel with newly developed tumor stroma. In approximately 70% of the cases tested, organoids transduced with shRNA against *Apc* (sh*Apc*) developed small but solid tumors over several weeks, while no tumor developed without *Apc* knockdown. With the co-introduction of sh*Pten* or sh*Trp53*, the development rate for solid tumors reached 100% and the size became larger, consistent

with earlier in vivo studies.^{14,52} Interestingly, sh*Apc*-driven tumors contained prominent mucus pools in the stroma, due to the intratumoral disruption of glands. Similarly, simultaneous augmentation of proliferation and apoptosis has been observed upon acute inactivation of *Apc* in the murine intestine.⁵³ As it proved to be mediated by the major Wnt-target gene *myc*,⁵⁴ these observations might be a reflection of *myc*'s dual roles.

3.2.4 | Large solid tumor with cysts

LSL-*Kras*^{G12D} organoids co-infected with lentiviruses encoding Cre and sh*Apc* rapidly gave rise to significantly large tumors with both cystic and solid components, consistent with a strong synergy between *Kras* mutation and *Apc* inactivation in vivo.^{45,46} Reflecting active angiogenesis, the surface of these tumors tended to be red and contained a huge amount of serous or hematogenous fluid inside cysts. Histologically, tumor glands were densely packed, while disrupted glands were rarely observed, suggestive of anti-apoptotic effects by *Kras*^{G12D}. Our experience in organoid-based tumorigenesis for the lung,⁵⁵ pancreas (Matsuura et al, *submitted*) and hepatobiliary tract (Ochiai et al, *submitted*) suggests that emergence of a

	Non-tumor		Tumor	
Classification	Matrigel plug	Non-tumorous nodule	Solid tumor	Large solid tumor with cysts
Macroscopic images (schematic view)				
Histological features				
Tubular glands	—	+	++	++
Disrupted glands	—	+	+	—
Dysplasia	—	—	+	+
Organoid-based carcinogenesis	← Control → ← <i>Kras</i> ^{G12D/+} → ← <i>shPten</i> → ← <i>shApc</i> → ← <i>shTrp53</i> →		← <i>shApc</i> + <i>Kras</i> ^{G12D/+} → ← <i>shApc</i> + <i>shPten</i> → ← <i>shApc</i> + <i>shTrp53</i> →	
Corresponding in vivo lesions	Normal	Aberrant crypt foci	Adenoma	Adenocarcinoma

FIGURE 3 Classification of subcutaneous nodules developed in nude mice. Tumors and non-tumors developed in the subcutis of nude mice are classified based on their macroscopic and microscopic features. The results from organoid-based carcinogenesis in murine intestinal cells (Ref. 17) are mapped in the lower row

cystic component is closely associated with *Kras*^{G12D}. Accordingly, future classification of the nodules might as well include “cyst” in the category of non-tumorous nodules.

4 | OTHER ORGANOID-BASED CARCINOGENESIS MODELS FOR THE INTESTINE AND COLON

Following our previous study,¹⁷ several studies reported modeling colon carcinogenesis with similar approaches.^{56–60} Although based on analogous concepts, they varied in many respects, including the methods for cell culture, genetic engineering and inoculation, as well as which organs or species were used. These studies are listed in Table 1.

4.1 | Liquid-air interface culture-based heterotopic carcinogenesis in nude mice

Minced tissue fragments were embedded in collagen gel, set in a culture insert, and exposed to serum-containing media on the bottom and to free air on the top.⁶¹ This setting, referred to as liquid-air interface culture, enables the generation of gradients in concentration of nutrients and oxygen, regenerating tissue structure in a configuration close to the intestinal mucosa. As both epithelial cells and stromal cells are maintained in culture, histological analysis can be directly conducted. Gene transduction was basically conducted in an inducible manner with tamoxifen or acute introduction of Cre.

Notably, delivery of Cre worked efficiently for adenovirus, but not for retrovirus, through brief exposure (approximately 30 minutes) to dissociated cells. Then, direct microinjection of the viral particles encoding shRNA or cDNA into the organoids was conducted, which successfully validated the pro-tumorigenic effects by 11p15.5 amplicon in *Apc*-null colon organoids.⁵⁶

4.2 | Colon organoid-based orthotopic carcinogenesis models in severe immunodeficient or syngenic mice

Colon organoids isolated from GEM harboring doxycycline (Dox)-inducible *shApc* were treated with Dox to knockdown *Apc*. To generate a niche for engraftment in the colon of host mice, mucosal damage had been induced in advance through oral administration of DSS. This procedure enabled development of benign adenomas in nude mice by *Apc* knockdown alone.⁶⁰ When further interbred with *Kras*^{G12D/+}; *Trp53*^{fl/fl} mice, their colon organoids, infected with adenovirus-Cre and treated with Dox to achieve triple mutations, gave rise to invasive carcinoma. Intriguingly, similar results were obtained for orthotopic inoculation in syngenic C57BL/6J mice, demonstrating significant augmentation of tumor engraftment by DSS. Moreover, metastases to the liver and the lung were also observed following injection to the spleen and tail vein, respectively.⁶⁰ In another study, organoids derived from colon adenoma in *Apc*^{min/+}; *Kras*^{G12D/+} mice were subject to further deletion of *Trp53* and *Smad4* through genome editing with CRISPR/Cas9. Orthotopic inoculation of these quadruple mutant organoids in severe immunodeficient NSG/NOG mice resulted in adenocarcinoma development with

TABLE 1 A list of representative studies on modeling colon carcinogenesis using organoid-based approaches

Organoids			Genetic alterations			Inoculation			Diagnosis	Reference
Species	Organ	Type	3-D culture	Genotypes	Transduction	shRNA/cDNA/Gene editing	Mouse	Site		
Mouse	SI	Normal	Matrigel (bilayer)	WT <i>Kras^{LSL-G12D/+}</i>	Lentivirus	<i>shApc, shTrp53, shPten</i> <i>shApc, Cre</i>	Nude	Subcutis	Adenoma-adenocarcinoma Aberrant crypt foci-adenocarcinoma Adenoma-adenocarcinoma Adenoma-adenocarcinoma	Onuma et al ¹⁷
Mouse	SI	Normal	Collagen (air-liquid interface)	<i>Apc^{low/flox}, Villin-Cre^{ER}</i> <i>Pten^{flox/flox}, Villin-Cre</i>	Tamoxifen +retrovirus	<i>shTrp53, shSmad4, Kras^{G12D}</i>	(in vitro)		Tubular adenomatous polyp-adenocarcinoma	Li et al ⁵⁶
	LI			<i>Apc^{low/flox}, Trp53^{low/flox}, Kras^{LSL-G12D/+}</i> <i>Apc^{low/flox}, Villin-Cre^{ER}</i>	Adenovirus	<i>Cre</i> <i>shTrp53, shSmad4, Kras^{G12D}</i>			Minimal dysplasia-adenocarcinoma High-grade focal dysplasia	
Human	SI	Normal	Matrigel (dome)	WT	Tamoxifen +retrovirus	<i>shTrp53, shSmad4, Kras^{G12D}</i>	NSG	Subcutis	Adenocarcinoma	Drost et al ⁵⁷
	LI				CRISPR/Cas9 (lipofection)	<i>APC, TP53, KRAS^{G12V}, SMAD4</i> <i>APC, TP53, KRAS^{G12V}, SMAD4</i> <i>APC, TP53, KRAS^{G12D}</i> <i>APC, TP53, KRAS^{G12V}, SMAD4</i>	NSG	Subcutis	Adenoma Invasive carcinoma Well differentiated carcinoma Poorly differentiated carcinoma	
Human	LI	Normal	Matrigel (dome)	WT	CRISPR/Cas9 (electroporation)	<i>APC</i> <i>APC, TP53, KRAS^{G12V}, SMAD4, PIK3CA^{E545K}</i>	NOG	Kidney subcapsule	None Adenocarcinoma	Matano et al ⁵⁸
	Adenoma			ND	Lentivirus	<i>CTNNB1^{S33Y}, KRAS^{G12V}, PIK3CA^{E545K}, shTP53, shSMAD4</i>			None	
Mouse	LI	Normal	Matrigel (dome)	<i>shApc</i>	CRISPR/Cas9 (electroporation)	<i>—</i> <i>TP53, KRAS^{G12V}, SMAD4, PIK3CAE^{S45K}</i>	Nude	Spleen	Adenocarcinoma with liver metastasis	O'Rourke et al ⁶⁰
	Adenoma			<i>shApc; Kras^{LSL-G12D}, p53^{R127H/+}, shSmad4</i>	Doxycycline +adenovirus	<i>Cre</i>		Colon (+DSS)	Benign tubular adenomas	
	Tumor (T3)			ND	Doxycycline +adenovirus	<i>Cre</i>		Colon (+DSS)	Carcinoma with submucosal invasion	
Mouse	LI	Adenoma	Matrigel (dome)	<i>Apc^{mlh1/+}, Kras^{LSL-G12D/+}, Villin^{Cre}, Lgr5^{DTR/eGFP}</i>	CRISPR/Cas9 (lipofection)	<i>Trp53, Smad4</i>	NSG	Colon	High-grade adenocarcinoma (liver, lung metastasis) Liver metastasis	De Sousa e Melo et al ⁵⁹
	Adenoma				Doxycycline +adenovirus	<i>Cre</i>		Colon (+DSS), spleen, tail vein	High-grade adenocarcinoma (liver, lung metastasis)	
	Tumor (T3)				Doxycycline +adenovirus	<i>Cre</i>		Spleen	Liver metastasis	
	Adenoma				CRISPR/Cas9 (lipofection)	<i>Trp53, Smad4</i>	NSG	Colon (+DSS)	Liver metastasis	
	Adenoma				CRISPR/Cas9 (lipofection)	<i>Trp53, Smad4</i>	NSG	Colon	Tumor development and liver metastasis	
	Adenoma				CRISPR/Cas9 (lipofection)	<i>Trp53, Smad4</i>	NSG	Portal vein	Liver metastasis	

CRC, colorectal cancer; DSS, dextran sulfate sodium; LI, large intestine; NSG/NOG, NOD scid gamma mouse (severe immunodeficient mouse); SI, small intestine; WT, wildtype.

spontaneous liver metastasis, which was inhibited by ablation of Lgr5⁺ cells, demonstrating critical roles of cancer stem cells in establishing metastasis.⁵⁹ These studies highlight the potential of the organoid-based adoptive transplant approaches in extending the research field, from tumorigenesis to metastatic progression and tumor immunity.

4.3 | Human organoid-based carcinogenesis models in severe immunodeficient mice

Organoid culture is also feasible for human intestinal cells.⁶² Normal small intestinal cells and colon cells were genetically engineered by CRISPR/Cas9 to reconstitute triple mutations in *APC*, *TP53* and *KRAS*, followed by depletion of factors or addition of inhibitors to enrich cells that acquired independency of the stem cell niches through introduced mutations.⁵⁷ Engineered organoids from intestine and colon developed adenomas and well-differentiated adenocarcinomas, respectively. Additional inactivation of *SMAD4* resulted in development of invasive carcinomas and poorly differentiated adenocarcinomas, respectively. In contrast, patient-derived colon adenoma organoids or *APC* inactivation in the colon organoids were not tumorigenic in the kidney subcapsule of NSG mice. These results suggest that *APC* inactivation alone in human cells is not sufficient to induce neoplastic changes even in NSG/NOG mice. Not surprisingly, CRISPR/Cas9-mediated introduction of mutations in *TP53*, *KRAS* and *PIK3CA* cooperated with *APC* inactivation to generate full-blown tumors and metastatic tumors from normal organoids and adenomas, respectively.⁵⁸ Taken together, these observations strongly suggest that human colon organoids can develop adenocarcinomas if only multiple mutations are introduced but not develop premalignant lesions, including ACF or adenomas.

5 | CONCLUSION

Each organoid-based colon carcinogenesis model overviewed herein has unique features. Accordingly, researchers will want to select the right model for their research, depending on the aim of their study, as well as taking into consideration that the time and cost that can be afforded. For example, models with murine colon organoids largely depend on the use of GEM with multiple mutations and place more emphasis on generating tumors or phenomena in the advanced stages.^{57,58,60} Although multiple intercrossing is required, these models might be most suitable for studies on tumor progression and metastasis and for preclinical trials. However, models with murine small intestinal organoids could detect genetic cooperation towards tumorigenesis with the highest sensitivity as preneoplastic lesions or tumors. Hence, validation of candidate genes, whether identified by a murine forward genetic screen⁶³ or in human CRC samples,¹⁹ is warranted.

Whereas organoids from inbred young laboratory mice of the C57BL/6 strain might be regarded as "clean" in terms of the homogenous nature of genomes and epigenomes, human organoids may

not be ideal in this regard because they might harbor genetic polymorphisms and accumulate mutations from environmental carcinogens and aging. Nonetheless, it is noteworthy that they for the first time enabled human models of carcinogenesis, which will provide valuable information not only for basic science but also for future practice of personalized medicine.

Taken together, organoid-based carcinogenesis models are promising tools in cancer research, which would likely substitute and complement in vivo mouse models to various degrees. Therefore, efforts toward establishing similar models for other types of cancer driven by different genetic aberrations are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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