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CANCER MODELS

Tailored mouse models

> transplanted organoid models can successfully mimic the full human adenoma-carcinoma-metastasis sequence *in vivo*

Traditional genetically engineered mouse models (GEMMs) or orthotopic transplantation models of colorectal cancer have several disadvantages that limit their utility in robust preclinical investigation: they poorly recapitulate advanced disease stages and the primary tumours form in the incorrect anatomical location. Two independent groups describe approaches to improve colorectal cancer modelling through orthotopic engraftment of syngeneic colon organoids engineered to harbour colorectal-cancer-predisposing mutations into mice.

To implant the organoids into the colons of mice, the two groups used different but complementary techniques. O'Rourke *et al.* used a non-invasive enema method that requires transient colonic injury through application of dextran sodium sulfate that exposes a niche for organoid engraftment. In contrast, Roper, Tammela *et al.* used a colonoscopy-guided procedure to inject organoids into the lamina propria of the colon, which has the advantage of not causing damage to the colon. These two techniques are superior to GEMMs with germline loss-of-function mutations in adenomatous polyposis coli (*Apc*) as tumours arise in the native environment of the colon and not in the small intestine.

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Both groups engineered colon organoids to contain mutations in *Apc*, *Kras* and *Trp53* (the three most frequently altered genes in human colorectal cancer). The triple-mutant organoids were then transplanted into the colons of syngeneic mice where they developed into focal primary tumours that progressed to locally invasive adenocarcinomas and spontaneous metastasis to the liver. The invasive tumours displayed a desmoplastic stromal reaction and invaded into the muscularis propria, reproducing stage II of human colorectal cancer; unlike similar GEMMs, which never develop past stage I owing to high overall tumour burden. Crucially, these mice had increased survival compared with GEMMs, affording more time for local vascular and lymph dissemination to be modelled. Importantly these findings show that transplanted organoid models can successfully mimic the full human adenoma-carcinoma-metastasis sequence *in vivo*.

To highlight the flexibility of the organoid transplant models, both groups tailored them to investigate colorectal cancer progression. O'Rourke *et al.* adapted their model to reproduce metastatic colorectal cancer growth whereby dissociated triple mutant organoids were injected into the splenic vein and formed tumour foci in the livers of mice. The authors then restored APC expression in the tumour cells, which caused inhibition of the WNT pathway and tumour cell differentiation in the form of large glands of postmitotic colon epithelium as well as regions of fibrosis and necrosis in the liver. Interestingly, longitudinal analysis of the tumours revealed that re-expression of APC was also associated with severe ascites, possibly as a result of ectopic functional colonic epithelium in the liver, highlighting potential undesirable effects of inhibitors of metastatic colorectal cancer.

Roper, Tammela *et al.* used their organoid transplant model to trace the lineage of LGR5$^+$ cells in established colon adenomas. Colon organoids derived from mice expressing a Cre-activatable fluorescent reporter to label LGR5$^+$ cells were generated and *Apc* was subsequently inactivated by CRISPR–Cas9. LGR5$^+$ cells increased in size relative to the total tumour area and contained more proliferating cells than LGR5$^-$ cell populations. This shows the cancer stem cell-like activity of LGR5$^+$ cells in accurate models of colon adenomas.

Lastly, a powerful application of these mouse models was shown by both groups, wherein orthotopic engraftment of patient-derived colorectal cancer organoids could faithfully recapitulate the histopathology of the patient tumour and progress to liver metastasis. Thus, the approach of orthotopically transplanting genotypically defined organoids is likely to uncover mechanisms that underlie the invasion and metastatic spread of colorectal cancer that might otherwise have been undetectable in standard mouse models.

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ORIGINAL ARTICLES O'Rourke, K. P. *et al.* Transplantation of engineered organoids enables rapid generation of metastatic mouse models of colorectal cancer. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3837 (2017) | Roper, J., Tammela, T. *et al. In vivo* genome editing and organoid transplantation models of colorectal cancer and metastasis. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3836 (2017)

FURTHER READING Fumagalli, A. *et al.* Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids. *Proc. Natl Acad. Sci. USA* **114**, E2357–E2364 (2017)