Oncogene-inducible organoids as a miniature platform to assess cancer characteristics

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Direct effects of oncogenic proteins or inhibitor treatments on signaling pathways are difficult to assess in transgenic mice. In this issue, Riemer et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201610058) demonstrate that oncogene-inducible organoids offer the experimental versatility of two-dimensional cell lines, while closely representing the in vivo situation.

The stepwise accumulation of genomic alterations, such as mutation or genetic loss of APC, KRAS, TP53, and SMAD4, was first described in colorectal cancer (CRC; Fearon and Vogelstein, 1990). The mutated oncogenes and/or tumor suppressors cooperatively drive cancer progression. Therefore, characterization of impacts of these genomic alterations on the various signal transduction pathways is crucial for the development of targeted therapy in CRC. Until recently, cancer cell lines and genetically engineered mouse models (GEMMs) have been the experimental workhorses. Because most cancer cell lines are derived from advanced cancers carrying multiple driver mutations, roles of isolated mutations or defined combinations of such mutations have been difficult to investigate in these cell lines. GEMMs offer this opportunity, but throughput of GEMM-based strategies is low, while detailed biochemical and cell-biological measurements in vivo are also complex to perform.

Organoids established from transgenic mice provide one possible solution to overcome these limitations. Organoid culture methods for mouse intestinal epithelial stem cells were first established in 2009 (Sato et al., 2009). Intestinal organoids contain Lgr5-positive adult stem cells that generate all intestinal cell lineages and recapitulate the architecture of proliferative crypt and differentiated villus units. Organoids can be expanded for long-term periods while remaining genetically and phenotypically stable. Since the first report on mouse intestinal epithelium, adult stem cell–based organoids have been developed for a variety of normal and malignant mouse and human epithelial tissues including colon, stomach, liver, and pancreas (Clevers, 2016). Importantly, organoids recapitulate architecture, functionality, and the genetic signature of their original tissues and can be used as disease models when they are directly established from the affected tissue. In this issue, Riemer et al. established organoids from transgenic mice carrying several CRC-related mutations and investigated the relationship between cancer phenotypes in organoids and signaling activities (Fig. 1).

Riemer et al. (2017) generated transgenic mice carrying doxycycline-inducible oncogenes, i.e., stabilized β-catenin (CTNNB1$^{\text{stab}}$), PIK3CA$^{\text{H1047R}}$, or both from a single expression cassette (CTNNB1$^{\text{stab}}$–PIK3CA$^{\text{H1047R}}$). In vivo, these two oncoproteins synergistically enhance the proliferation of intestinal epithelial cells. For a detailed analysis in vitro, the authors converted intestinal epithelial stem cells into organoids (Fig. 1 A). Transcriptome analysis after the induction of CTNNB1$^{\text{stab}}$, PIK3CA$^{\text{H1047R}}$ alone, or both combined suggested that apoptosis-related genes were suppressed by the oncoproteins, whereas genes related to DNA replication and cell cycle progression were up-regulated (Fig. 1 B). In PIK3CA$^{\text{H1047R}}$-induced organoids, metabolic signatures (for glycolysis and oxidative phosphorylation) were strongly induced. As expected, the intestinal Wnt-driven stem cell signature was seen specifically in CTNNB1$^{\text{stab}}$-induced organoids. These transcriptome-based results were validated by in vitro functional assays (Fig. 1 B), leading to the conclusion that both oncoproteins promote proliferation and repress anoikis of intestinal epithelial cells, whereas the role of β-catenin in the maintenance of intestinal stem cell function is not compensated by the induction of PIK3CA$^{\text{H1047R}}$.

Riemer et al. (2017) could not confirm previous studies showing that oncogenic PIK3CA would contribute to invasiveness of CRC cells cooperatively with activated β-catenin (Samuels et al., 2005; Leystra et al., 2012). Although Riemer et al. (2017) raised the possibilities that CTNNB1$^{\text{stab}}$- and PIK3CA$^{\text{H1047R}}$-coexpressing organoids lacked a chemotactic response to growth factors or migrated as a collective form, they concluded that CTNNB1$^{\text{stab}}$ and PIK3CA$^{\text{H1047R}}$ oncoproteins are not sufficient on their own to induce invasiveness or epithelial–mesenchymal transition in intestinal epithelial cells. The authors then quantified attachment of organoids to culture dish surface and motility in 2D. Although organoids tended not to attach to the plate surface when CTNNB1$^{\text{stab}}$ or PIK3CA$^{\text{H1047R}}$ were induced alone, organoids in which both oncoproteins were induced frequently attached and spread in 2D. Finally, the authors performed a nonbiased functional pathway analysis using a panel of pharmaceutical inhibitors against MEK (AZD6244), PI3K (GDC0941), AKT (MK2206), mTOR (Rapamycin and Torin 1), and GSK3β (Fig. 1 C). This type of analysis would be difficult to perform in experiments using transgenic mice. Consistent with a previous study showing that Rapamycin is effective in Apc-deficient intestinal cells in mice (Faller et al., 2015),

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 216 No. 6 1505–1507
https://doi.org/10.1083/jcb.201704014

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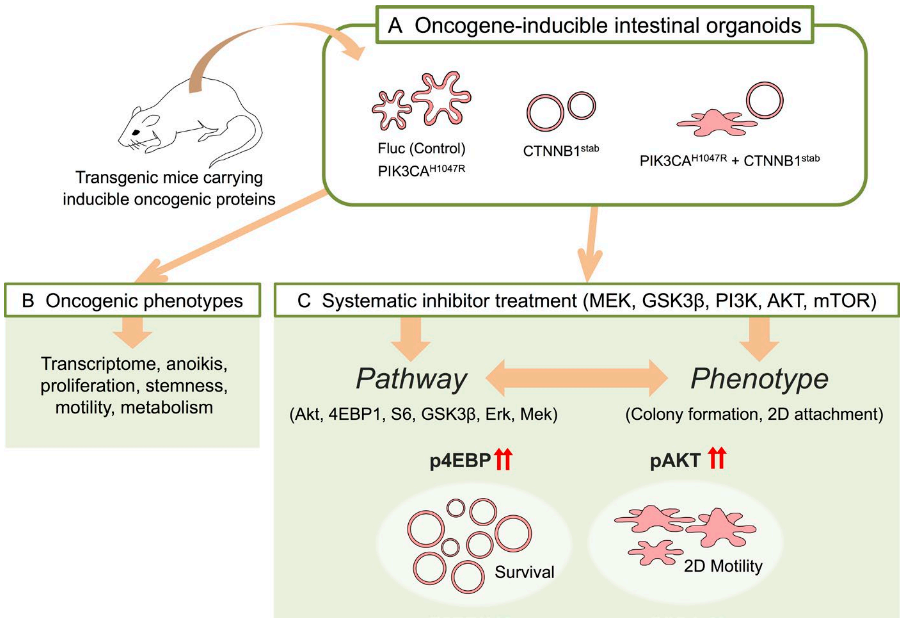
A Oncogene-inducible intestinal organoids
Transgenic mice carrying inducible oncogenic proteins
Fluc (Control) PIK3CA^{H1047R}
CTNNB1^{stab}
PIK3CA^{H1047R} + CTNNB1^{stab}
B Oncogenic phenotypes
Transcriptome, anoikis, proliferation, stemness, motility, metabolism
C Systematic inhibitor treatment (MEK, GSK3β, PI3K, AKT, mTOR)
Pathway
(Akt, 4EBP1, S6, GSK3β, Erk, Mek)
Phenotype
(Colony formation, 2D attachment)
p4EBP ⇈
pAKT ⇈
Survival
2D Motility

Figure 1. **Schematic of the approach to assessing cancer characteristics.** (A) Intestinal organoids are established from transgenic mice carrying inducible oncoproteins. Their morphological features vary depending on the oncogenic proteins. (B) Oncogenic phenotypes of the organoids can be analyzed by well-established in vitro assays, such as gene expression, proliferation, metabolic, and apoptosis assays. (C) Organoids carrying inducible proteins enable nonbiased pathway analysis in the context of (combinations of) pathway inhibitors.

organoids producing CTNNB1^{stab} alone were highly sensitive to Rapamycin. Of note, these mutations both result in an activated Wnt pathway. The sensitivity to Rapamycin of CTNNB1^{stab} organoids was canceled by the acquisition of PIK3CA^{H1047R}, suggesting that Rapamycin would be effective only at the initial stage of colon tumors, i.e., when only Apc is mutant. Riemer et al. (2017) found other novel relationships between the pharmaceutical inhibitors and the oncogenic mutants. For example, AZD6244 and Torin 1 significantly suppressed the growth of organoids produced by CTNNB1^{stab} alone and by both oncoproteins together. GDC0941 was highly effective for organoids producing CTNNB1^{stab} alone.

Riemer et al. (2017) further examined the phosphorylation levels of signaling molecules (AKT, 4EBP1, S6, GSK3β, ERK1/2, and MEK1/2) and oncogenic phenotypes, such as colony formation and cell attachment, upon inhibitor treatment (Fig. 1 C). Although phosphorylation status was not significantly changed by the CTNNB1^{stab} alone, induction of both CTNNB1^{stab} and PIK3CA^{H1047R} mutants resulted in increased phosphorylation levels of AKT, GSK3β, 4EBP1, and S6. After the inhibitor treatment, the authors observed bidirectional negative feedback between AKT and ERK pathways. Interestingly, their data suggested that oncogenic activation of β-catenin might play a key role in AKT to ERK signaling feedback in intestinal cells. Furthermore, induction of both proteins in intestinal organoids resulted in uncoupled signaling between AKT and downstream mTORC1 events, even though mTORC1 activity is believed to be regulated by the PI3K–AKT pathway. These data may be helpful to understand the molecular mechanism of drug resistance caused by particular oncogenic mutants. In particular, recent studies have demonstrated that mTORC1 activity may predict drug efficacy (She et al., 2010; Corcoran et al., 2013). 4EBP1, one of the downstream effectors of mTORC1, may represent a key molecule for organoid attachment as predicted when phosphorylation status was compared between the oncogenic combinations.

Because organoids can be established from various epithelial tissues in both mouse and human, the strategy is applicable to other types of cancers. A broad variety of GEMMs for human carcinomas exists (Kersten et al., 2017). Organoids generated from these models could be used for the mechanistic studies that use high throughput drug screening, and then followed up with validation studies in the pertinent mouse model.

It should be mentioned that similarly defined cancer models can be engineered directly from human organoids by CRISPR/Cas9-mediated gene editing. Two studies (Drost et al., 2015; Matano et al., 2015) have independently recapitulated CRC by introducing sequential mutations of genes that are commonly altered in CRC, i.e., APC, KRAS, TP53, SMAD4, and PIK3CA. Subsequent transplantation of these organoids into immunodeficient mice has allowed a detailed study of migration and metastasis in vivo (Fumagalli et al., 2017).

In conclusion, Riemer et al. (2017) show that oncogene-induced organoids derived from GEMM can be used as exquisite tools to understand oncogene-related signal pathways and cancer-related phenotypes in 3D organoids in vitro (Fig. 1). This strategy will facilitate the study of individual cancer-related gene changes and genetic interactions between these and provide the opportunity to accelerate the development of effective targeted cancer therapies.

Acknowledgments

Work in the authors' laboratory is partly supported by MKMD grant from Netherlands Organization for Scientific Research (NWO-ZonMw; 114021012) to H. Clevers and a "Sta op tegen Kanker" International Translational Cancer Research Grant to H. Clevers. Stand Up to Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research. Y. Tsukamoto is partly supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (#15KK0351 and #15K08406).

The authors declare no competing financial interests.

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