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Short review

Investigation of early neoplastic transformation and premalignant biology using genetically engineered organoid models

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ABSTRACT

Organoid modeling is a powerful, robust and efficient technology faithfully preserving physiological and pathological characteristics of tissues of origin. Recently, substantial advances have been made in applying genetically engineered organoid models to study early tumorigenesis and premalignant biology. These efforts promise to identify novel avenues for early cancer detection, intervention and prevention. Here, we highlight significant advancements in the functional characterization of early genomic and epigenomic events during neoplastic evolution using organoid modeling, discuss the application of the lineage-tracing methodology in organoids to study cancer cells-of-origin, and review future opportunities for further development and improvement of organoid modeling of cancer precursors.

1. Introduction

Tumorigenesis is a highly complex and lengthy biological process, involving both intrinsic (e.g, genetic backgrounds, somatic mutations, epigenomic dysregulation), and extrinsic elements (e.g., environmental factors, inflammation). Aided by technological and methodical breakthroughs, our understanding of the biological hallmarks of established cancers has substantially progressed over the last 1–2 decades [1–3]. However, in comparison, the investigation and characterization of early biological alterations during the initial steps of neoplastic transformation are still limited for most cancer types. In particular, direct functional and phenotypic modeling of human premalignant lesions (e.g., dysplasia, metaplasia) remains scarce.

Clearly, high-resolution characterization of early alterations during neoplastic evolution and transformation holds the key to establishing the mechanistic basis underlying tumorigenesis. Moreover, these efforts have the potential to significantly advance the development of methods for early detection, intervention and prevention of human cancer. However, functional modeling of early events driving neoplastic evolution remains challenging, largely due to the difficulty of generating and maintaining cancer cells-of-origin *in vitro* in a physiological condition. In addition, cells-of-origin have not been precisely defined for some cancer types. Likewise, it is notoriously challenging to directly manipulate cancer precursor lesions, which are biologically and pathologically transitional in nature. Indeed, viable and valid model systems recapitulating the unique intermediate, transitory state of precursor lesions have been lacking for the majority of human cancer types.

One way to overcome these major obstacles is through the use of genetically engineered mouse models (GEMMs) [4]. However, generation and characterization of GEMMs are time-consuming and expensive. Indeed, genetic engineering of driver events in the correct cells-of-origin is laborious and difficult to scale up. Furthermore, because of key discrepancies between human and mouse anatomy, GEMMs cannot faithfully model certain human cancers. In addition, murine precursor lesions are miniature in size and quantity, limiting their use for biological interrogation.

Organoid modeling provides a robust and powerful alternative to study premalignancy and tumorigenesis. Indeed, the organoid system recapitulates and maintains genetic, biological and phenotypic characteristics of the corresponding tissues of origin [5]. Moreover, organoid culturing has made it feasible and efficient to

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genetically manipulate cancer cells-of-origin directly from human samples. To date, many studies have established cancer organoids to investigate tumor biology and cancer gene functions. For example, one of the earliest works has successfully transformed normal murine intestinal organoids into colon cancer upon introduction of genetic mutations targeting Apc, Tp53, Krad and Smad [6]. This finding was confirmed by two independent studies of human intestinal organoids using CRISPR/Cas9-mediated genome editing of APC, $KRAS^{G12V/D}$, SMAD4, $PIK3CA^{E545K}$ and TP53 [7,8]. In fact, CRISPR/Cas9-based genome editing of normal organoids has led to successful neoplastic transformations of many different human tissues, including: brain, breast, ovary, liver, pancreas and stomach [9,10]. Moreover, direct organoid culture of fresh patient tumor specimens has been used to identify therapeutic strategies through correlating drug sensitivities to genomic alterations. These findings have been summarized and discussed extensively elsewhere [5,11]. The present work is focused on reviewing functional genomic and epigenomic investigations of premalignant lesions and neoplastic evolution using organoid modeling (Fig. 1).

2. Functional characterization of early genomic drivers of cancer precursors using organoid modeling

Germline mutations in mismatch repair genes (MMRs), most frequently involving MSH2 and MLH1, cause Lynch syndrome and hereditary colorectal cancer [12,13]. While the genome biology of MMR mutations in established colorectal tumors has been extensively characterized, their functional significance during the earliest steps of neoplastic evolution is relatively less understood. To address this, both normal intestinal and adenoma (precursor lesion) organoids were developed from Msh2-knockout mice for longitudinal monitoring [14]. Notably, normal Msh2-knockout organoids exhibited increased transient, cyst-like growth, which was independent of R-spondin, a key Wnt pathway activator necessary for the survival and self-renewal of normal intestinal stem cells [15–17]. Importantly, months prior to tumor initiation, normal Msh2-deficient intestinal tissue contained precursor cells that formed organoids with higher microsatellite instability signature, cyst-like growth and high proliferation rates. The organoid modeling data of hereditary colonic premalignancy thus identifies alterations proceeding long before tumor onset in the MMR-deficient intestine.

Compared with this relatively rare type of hereditary colorectal cancer, the premalignant state of sporadic, non-hereditary colorectal cancer is distinctively heterogeneous, comprising precursor lesions such as tubular adenomas, traditional and sessile serrated adenomas [18]. These precursors differ in histology, pathology as well as genomic aberrations. For example, sessile serrated adenomas often harbor the hotspot mutation of the BRAF (V600E) oncogene and the DNA hypermethylation phenotype [19]. To investigate the biology of these different colonic premalignant lesions, Fessler et al. generated organoids directly from human tubular adenoma samples [20]. They also modeled the serrated pathway by introducing a $BRAF^{V600E}$ mutation through genome editing. Using these precursor-derived organoids, they showed that Transforming growth factor-b (TGFb) signaling (a known driver for colorectal cancer) triggered distinct responses in different premalignant organoids. Specifically, TGFb caused apoptosis in tubular adenoma organoids, while it induced a mesenchymal phenotype in the $BRAF^{V600E}$-mutated organoids. Relatedly, Sato's group established human organoids from traditional serrated adenoma samples and characterized their genomic aberrations and phenotypes [21]. Moreover, introduction of EIF3E-RSPO2 fusions into colon organoids using CRISPR-Cas9-mediated chromosome engineering allowed functional and phenotypic modeling of the disease progression of traditional serrated adenomas. Upon orthotopic implantation, $EIF3E\text{-}RSPO2^{+}$ organoids (with $BRAF^{V600E}$ and TP53-mutant background) efficiently formed tumors histopathologically resembling sessile serrated adenomas, establishing the central role of these driver events in the precancerous stages of colonic tumorigenesis.

Premalignant lesions of pancreatic ductal adenocarcinoma (PDAC) are analogously heterogeneous, with major subtypes (based on the histopathology) including pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasms (IPMNs) [22,23]. This is further complicated by the presence of distinct cells-of-origin in these lesions: ductal and acinar epithelial cells. At the genomic level, PanIN is dominated by $KRAS^{G12D}$ mutations, while $GNAS^{R201C}$ mutations occur more frequently in IPMN lesions (either alone or together with $KRAS^{G12D}$) [24,25]. To investigate how distinct precancerous lesions affect cancer development, both pancreatic ductal and acinar organoids have been generated from human stem cells [26]. Interestingly, $KRAS^{G12D}$ and $GNAS^{R201C}$ expression in these pancreatic organoids induced different phenotypes dependent on the cell types tested. Notably, *in vivo* data showed that only $KRAS^{G12D}$ but not $GNAS^{R201C}$ was able to trigger premalignant changes. Moreover, $KRAS^{G12D}$ also induced acinar-to-ductal metaplasia, a key pathological process known to predispose the pancreas to malignant transformation. Another related study generated ductal organoids directly from human pancreatic tissue, followed by lentiviral-mediated overexpression of $KRAS^{G12D}$ and CRISPR/CAS9-based knockout of CDKN2A, SMAD4 and TP53 [27]. The combination of these genetic modifications led to the development of precursor lesions resembling human PanINs both *in vitro* and upon orthotopic transplantation. These results are consistent with observations obtained from $KRAS^{G12D}$ GEMMs for pancreatic cancer [28,29].

Barrett's esophagus (BE), a specialized columnar metaplasia that develops in response to chronic gastroesophageal reflux, is recognized as the precursor lesion of esophageal adenocarcinoma (EAC). Genetically, multiple recurrently mutated genes are present in BE tissues (e.g., TP53, CDKN2A and ARID1A) [30,31]. The risk of EAC in BE patients is increased 11–30-fold [32]. BE thus appears to serve as an ideal pre-malignant model for the investigation of the step-wise neoplastic evolution of the esophagus. However, the molecular mechanisms underlying the BE-associated neoplastic evolution remain largely elusive. One of the most crucial questions, identification of the primary driver(s) for the malignant transformation of BE into EAC, remains unresolved. This shortcoming is partly due to difficulties in performing molecular research into BE, largely because *in vitro* models representing this unique pathological transition have been lacking. Overcoming this major obstacle, we have established and propagated 3D organoids derived from human BE tissues [33]. More importantly, we performed CRISPR/Cas9 genome editing and introduced two frame-shift mutations to the APC gene, a key tumor suppressor whose down-regulation promotes BE-associated neoplasia [34,35]. Following APC gene knockout, BE organoids grew significantly faster than did wildtype organoids transfected with the empty vector. Moreover, the organoid-forming efficiency and lifespan (total number of passages) of single-cell dissociated APC^{ko} organoids were both higher than in wildtype control organoids. In addition, APC^{ko} BE organoids exhibited a more complex glandular structure. Histologically, wildtype BE organoids showed a single-layered epithelium with regular architecture and none/mild cytological atypia with retained nuclear polarity. In contrast, APC^{ko} organoids showed a multilayered epithelium characterized by architectural irregularity and cytological atypia, with loss of nuclear polarity and increased Ki67 staining. Similarly, BE organoids derived from the transgenic L2-IL-1B BE mouse model have also been developed for the investigation of different neoplastic-promoting factors including TLR2

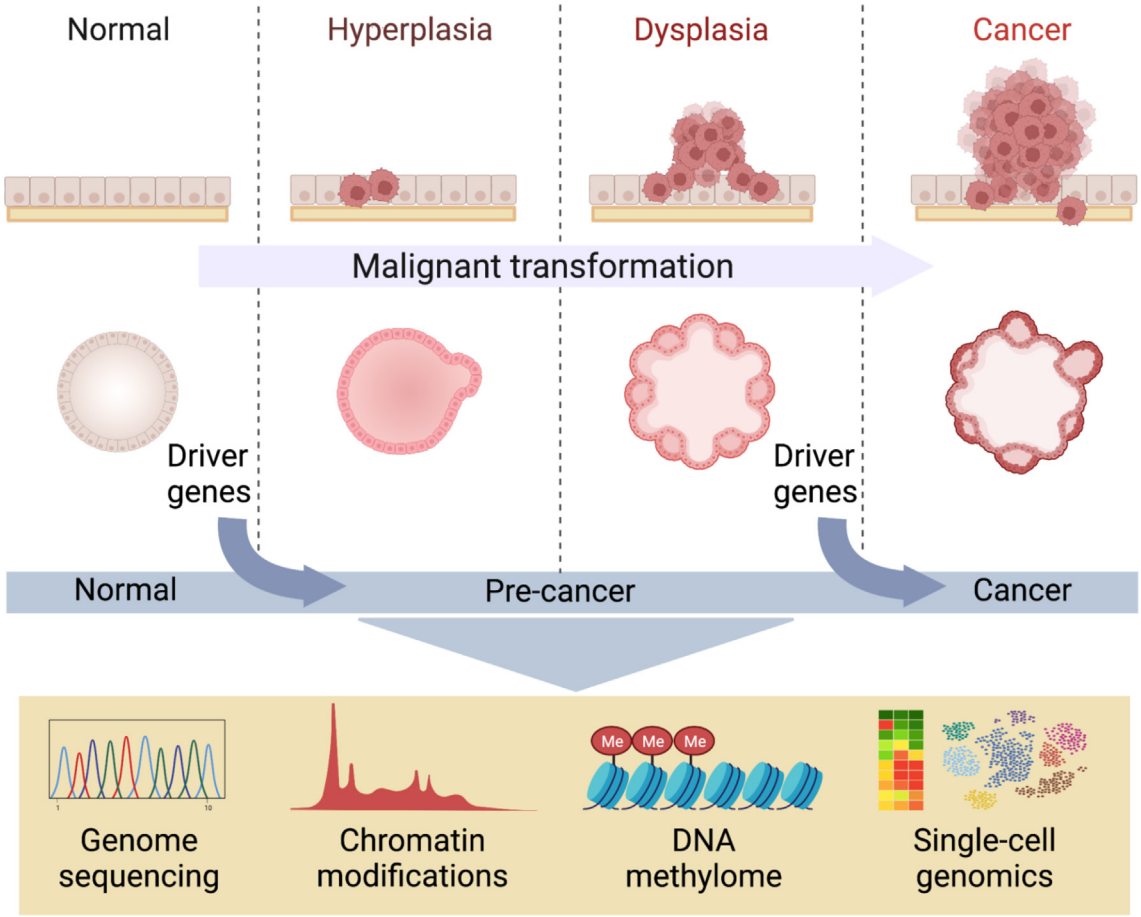

Fig. 1. Genomic and epigenomic characterization of early neoplastic transformation and premalignancy using organoid modeling. Precursor-derived organoids and genetically engineered premalignant organoids serve as versatile models for characterization of both genomic and epigenomic alterations during malignant transformation.

and CCK2R [36]. These results together highlight BE organoids as a viable *in vitro* model system for the functional interrogation of driver genes contributing to BE-associated neoplastic evolution.

Our group recently also reported the generation and establishment of human gastroesophageal junction (GEJ) organoids to characterize the early neoplastic transformation at the GEJ [37]. Compared with other gastroesophageal cancers, those arising at the GEJ are particularly aggressive, lack effective targeted therapies, and carry a dire prognosis. To vigorously investigate GEJ malignant transformation, we generated TP53/CDKN2A dual-knockout (TP53/CDKN2A^{DKO}) human GEJ-derived organoids edited using CRISPR-Cas9 mediated engineering. Notably, dual-knockout organoids grew faster, became larger, and exhibited *de novo* intestinal, metaplastic and dysplastic morphology. Moreover, dual-knockout organoids consistently underwent xenograft growth *in vivo*, with the histopathology also resembling intestinal metaplasia and dysplasia, an established precursor condition for GEJ cancer.

Interestingly, similar observations have been made in murine gastric organoids upon dual-knockout of TP53 and CDKN2A, which promoted gastric premalignancy [38]. In fact, intestinal metaplasia of the stomach is also considered a precancerous lesion to intestinal-type gastric cancer. In another study characterizing the molecular mechanisms of this gastric premalignant condition, a human intestinal metaplasia model was established using gastric organoids derived from human-induced pluripotent stem cells (hiPSCs), followed by functional analyses of the CDX2 gene, which has been implicated in intestinal metaplasia in mouse models [39]. Notably, inducible overexpression of CDX2 led to intestinal phenotypes and turned on the gene signature of intestinal metaplasia.

While the majority of organoid modeling of premalignant states has been focused on gastrointestinal (GI) organs, other non-GI cancer precursors started to emerge recently, such as neoplasia of the prostate [40], breast [41] and lung [42]. For example, using murine prostate organoids, the role of ERG (a driver gene of prostate cancer) was investigated at the earliest stages of prostate transformation. Specifically, a shorter form of ERG was cloned from TMPRSS2-ERG^{+} prostate cancer cells and was ectopically expressed in normal murine prostate organoids. Overexpression of shorter ERG deregulated prostate cell proliferation and differentiation, and promoted the survival and growth of prostate cells under growth factor restrictions. Mechanistically, overexpression of the shorter-form of ERG represses the canonical Wnt pathway while triggering the accumulation of DNA double-strand breaks via the degradation of Nkx3.1, a tumor suppressor in prostate cells. In normal breast epithelial organoids, CRISPR-Cas9 mediated co-inhibition of P53, PTEN and RB1 caused incomplete neoplastic transformation, as evidenced by increased proliferation *in vitro* and ~80 % success rate for *in vivo* implantation. Notably, mutation of an additional tumor suppressor gene, NF1, further increased tumor formation rate *in vivo*, indicating a near complete malignant transformation [41].

Together, as summarized in Fig. 2, these premalignant organoid models have provided important insights into the precancerous biology of different tissue types and established causal effects of genomic drivers during early stages of cellular malignant evolution.

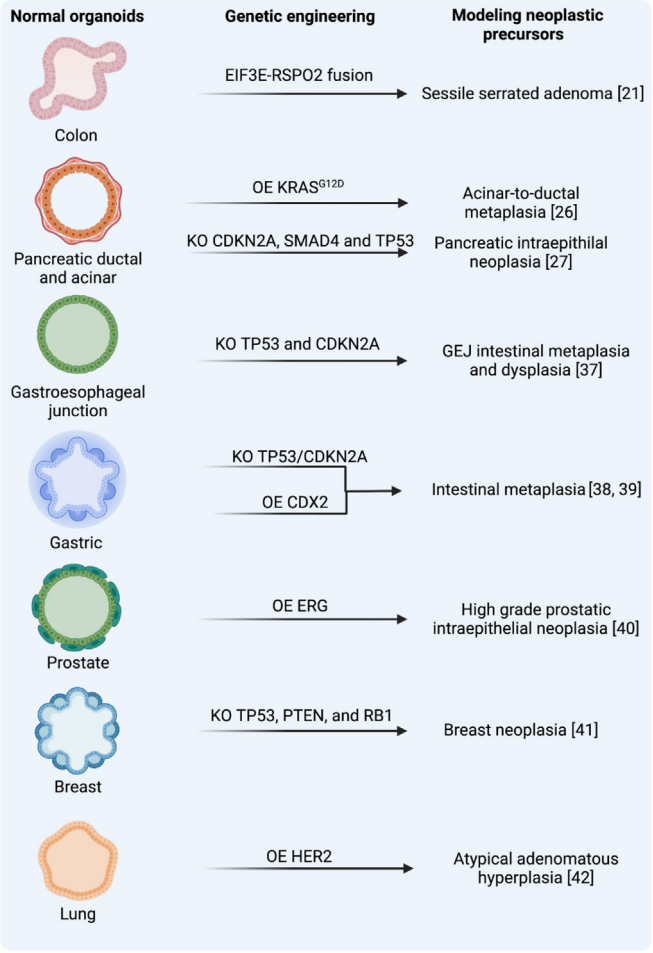

Fig. 2. Genetically-engineered premalignant organoid models. Premalignant organoids can be derived from normal organoids undergoing forward oncogenic transformation upon genetic-engineering-based introduction of cancer drivers. KO, knockout; OE, overexpression; GEJ, gastroesophageal junction.

3. Organoid modeling reveals (*epi*)genomic mechanisms during early neoplastic transformation

Although it has become well-recognized that cancer is driven by both genomic and epigenomic abnormalities, whether and how these two forms of forces interplay and cooperate during early tumorigenesis is much less understood. For example, although DNA hypermethylation at CpG island (CGIs) promoters represents perhaps one of the most prominent epigenetic aberrations in cancer, when and how it is established during neoplastic transformation, as well as its functional interaction with genomic drivers, remains unclear. Notably, *de novo* hypermethylation of CGI promoters also occurs spontaneously in normal aging cells, further complicating the study of the functional significance of this type of epigenetic change in cancer. To address these questions, Tao and colleagues modeled disease progression through the early colonic neoplastic transformation using an organoid platform and identified the role of spontaneous DNA hypermethylation in establishing a permissive epigenome for $BRAF^{V600E}$-driven tumorigenesis [43]. Specifically, promoter DNA hypermethylation spontaneously arose in a progressive, clock-like manner during long-term culture (12–14 months) of colon organoids. This led to the epigenetic silencing of the Wnt pathway, generating a progenitor-like, de-differentiated cellular state. Importantly, these epigenetic deregulations caused aged (long-term cultured) organoids to be exceedingly more susceptible to malignant transformation by $Braf^{V600E}$ than younger ones. In fact, it only took $Braf^{V600E}$ two weeks to transform old organoids, compared with 5 months for young organoid counterparts. Moreover, these aging-like epigenetic defects were phenocopied by genomic knockout of four key hypermethylation-targeting genes. These findings highlight the complex interplay and cooperation between CGI hypermethylation and driver mutations in the earliest stages of colonic tumorigenesis. Congruently, in human colon samples, both $BRAF^{V600E}$ and CGI hypermethylation are found in early precancerous lesions [44].

In a stepwise tumorigenesis organoid model of human retinoblastoma, whole-genome bisulfite sequencing identified thousands of differentially methylated regions (DMRs) in retinoblastoma organoids, which were distributed genome-wide and enriched in noncoding regions [45]. DNA methylation levels of key retinoblastoma-relevant driver genes, including SYK, RXRG, MKI67 and CCNE2, were reduced in tumor organoids, accompanied by increased expression of these factors. Pathway enrichment analysis of genes within DMRs implicated the activation of Axon guidance, Pathways in cancer, and the PI3K-Akt signaling during the tumorigenesis of retinoblastoma.

We also interrogated DNA methylome changes in early GEJ dysplastic organoids upon dual-knockout of TP53/CDKN2A, and identified thousands of differentially methylated CpGs and DMRs between control and $TP53/CDKN2A^{DKO}$ organoids[37]. Since DNA hypomethylated regions contain regulatory elements associated with the binding of transcription factors (TFs), we searched for enriched TF-recognition motif sequences in hypomethylated DMRs of $TP53/CDKN2A^{DKO}$ organoids [46,47]. This motif sequence enrichment analysis, coupled with RNA-seq data and functional assays, identified and validated FOXM1 as a key master regulator TF activated by dual-knockout of TP53/CDKN2A. Indeed, FOXM1 binding motif was strongly enriched in hypomethylated DMRs of $TP53/CDKN2A^{DKO}$ organoids. FOXM1 expression was upregulated by dual-knockout of TP53/CDKN2A and overexpressed in EAC/GEJ tumor samples compared with normal tissues. Functionally, FOXM1 promoted neoplastic evolution of GEJ organoids. These data identified crucial epigenomic changes occurring during early GEJ tumorigenesis.

Relatedly, another master regulator TF, SOX2, was characterized during the neoplastic transformation of esophageal squamous cell carcinoma (ESCC) using squamous organoids [48]. SOX2 is a well-recognized oncogenic TF in established ESCC and is often genomically amplified in this cancer type [49–52]. Nevertheless, the manner in which its genomic occupancy and epigenomic activity are deregulated during the malignant transformation of ESCC remains ill-defined. To address this critical knowledge gap, engineered murine organoids were developed that spanned from normal squamous esophagus, pre-neoplasia (dual-knockout of p53/p16), to full-blown ESCC upon Sox2-overexpression. Using ChIP-seq, ATAC-Seq and RNA-Seq, the genomic occupancy of Sox2 and its transcriptional landscape was characterized during the neoplastic transformation of esophageal squamous epithelium. Notably, in the background of p53/p16 inactivation, Sox2 overexpression promoted extensive chromatin remodeling, gained tens of thousands of binding sites, and established hundreds of *de novo* super-enhancers. Interestingly, Sox2 overexpression also activated endogenous retroviruses, induced expression of double-stranded RNA, and created a unique survival addiction on ADAR1, an RNA editing enzyme. These results shed important light on the malignant transformation of esophageal squamous epithelium and identify early epigenomic dysregulation with potentially targetable vulnerabilities.

The epigenomic and transcriptomic characteristics of gastric premalignancy were also recently investigated using metaplastic and dysplastic organoid lines established from mouse stomach cor-

pus [53]. Single-cell RNA-Seq (scRNA-seq) showed that the metaplastic and dysplastic organoids separated completely, underscoring distinct transcriptomes between the metaplastic and dysplastic cellular states. Interestingly, while metaplastic organoids exhibited low transcriptomic heterogeneity, the dysplastic counterpart consisted of a small, metaplastic-like subpopulation and a major, dysplastic-specific subcluster. Differential expression analysis between metaplastic and dysplastic clusters identified known genes that support these two premalignant stages, including Wfdc2, Mal2, Gpx2, Cd44, etc. These single-cell analyses thus directly identified transcriptomic heterogeneity in different stages of gastric precursor lesions.

4. Mapping cancer cell-of-origin using the organoid platform

Mapping accurately the cells-of-origin of human cancer is imperative for understanding the precise mechanistic basis of step-wise neoplastic evolution. However, identifying originating cells of many different cancer types remains challenging. For example, with respect to retinoblastoma, prior studies using mouse models have proposed either amacrine, horizontal cells, or Müller glial precursor cells as tumor-initiating cells [54–56]. However, a notable weakness is that these retinoblastoma mouse models lack important human disease characteristics. On the other hand, either human cone precursors or retinal progenitors have been identified as originating cells of retinoblastoma [57–61]. These discrepancies are partially attributable to the lack of a robust and sustainable human disease model. To tackle these problems, a recent study genetically engineered human embryonic stem cells (hESCs) with homozygous RB1 nonsense mutation (R320X) or knockout, which were further induced to differentiate progressively towards retinoblastoma organoids [45]. Importantly, scRNA-seq of retinoblastoma organoids followed by a pseudotime trajectory analysis (a computational method quantifying the relative progression of the underlying biological process at the single cell level) highlighted ARR3^{+} maturing cone precursors at the branch point, indicating the multipotent potential of these cells. This ARR3^{+} cluster was sequentially followed by retinoma-like and retinoblastoma cells, which was validated by RNA velocity analysis. These results suggested the ARR3+ maturing cone precursor as the initiating cells of retinoblastoma. In a related, more recent scRNA-seq study of retinoblastoma organoid models, proliferating cone precursors (RXRγ^{+}Ki67^{+}) were instead proposed as the tumor-originating cell population [62]. The disparity might be due to patient-specific genetic background, which can influence retinoblastoma initiation and development. Indeed, in addition to an hESC line, the latter investigation also characterized retinoblastoma organoids from a patient-specific induced pluripotent line.

In PDAC, two distinct cells-of-origin have been proposed: ductal and acinar epithelial cells. Notably, in human stem cell-derived pancreatic organoids, PDAC driver GNAS^{R201C} induced cystic growth more effectively in ductal than acinar organoids. Conversely, the other major PDAC driver, KRAS^{G12D}, promoted tumorigenesis much more potently in acinar than ductal organoids. These contrasting effects are largely congruent with prior observations from PDAC GEMMs [28,29]. These data underscore the utility and value of these organoid models representing different PDAC cells-of-origin.

Another prominent example of characterizing cancer cells-of-origin facilitated by organoid models is from colon cancer. Initial *in vivo* lineage tracing of GEMMs showed that only in Lgr5^{+} intestinal stem cells, but not other cell types, could Apc inactivation induce intestinal adenomas, thereby establishing Lgr5^{+} intestinal stem cells as the tumor-initiating population [63]. This similar lineage-tracing strategy was later employed in organoid modeling to study cells-of-origin in human colon cancer [64]. In human primary colon organoids, lineage-tracing of an inducible Cre knock-in allele of LGR5 established and confirmed the self-renewal and differentiation capability of LGR5^{+} tumor-initiating cells. The organoid system allowed for further genetic manipulation, and revealed that depletion of LGR5^{+} tumor-originating cells led to only short-term regression. Strikingly, tumor recurrence was found to be driven by re-emerging LGR5^{+} cancer-initiating cells, replenished by other more differentiated cancer cells. These findings not only provide important insights into the identity and plasticity of cancer cells-of-origin, but also underline the organoid technology as a superb model for studying cancer initiation and hierarchy during the malignant transformation.

5. Limitations and future opportunities

The above findings have demonstrated that genetically engineered organoid models serve as a powerful platform for the investigation of early neoplastic transformation and premalignant biology. Nevertheless, substantial limitations and bottlenecks remain to be resolved. For example, generation, culture and maintenance of organoids directly from human precursor samples remain challenging, and current studies are mostly restricted to GI lesions (e.g., adenoma of the colon, BE). There is also a lack of organoid modeling of pediatric premalignant tissues. The development and establishment of organoids from other different human precancerous lesions entails not only a precise definition of cancer cells-of-origin, careful optimization of culture conditions (growth factors, niche factors, etc.), but also rigorous phenotypic and functional validation following initial organoid growth.

Important opportunities also exist for using organoids to model cancer-related environmental factors, such as oncogenic pathogens (Epstein-Barr virus, human papillomavirus, hepatitis B virus, Helicobacter pylori (*H.* pylori), etc.). This has already been explored in the context of *H.* pylori-associated gastric cancer. Indeed, oncogenic strains of *H.* pylori stimulated potent inflammatory responses and epithelial hyper-proliferation in normal gastric organoids [65–68]. Other prominent examples can be found from a Salmonella-associated gallbladder organoid model and certain E. coli-associated colon organoid models [69]. Nevertheless, a number of prominent and prevalent oncogenic viruses await characterization. By the same token, organoid modeling can be extended to investigate both short- and long-term effects of lifestyle risk factors, including tobacco, alcohol, diet and obesity.

Although evolution and transformation of pre-neoplastic cells has largely been opined and investigated as an intrinsically-driven process, the premalignant microenvironment is increasingly being recognized to play a key role in regulating the biology of precancerous cells. For example, recent genomic and epigenomic profilings have identified a prominent interplay between precancerous cells and local immune population, and provided compelling evidence that precursor lesions already evolve to evade immune surveillance and attack during the earliest stages of tumorigenesis [70,71]. Therefore, significant potentials are present for exploiting organoid modeling to investigate the premalignant microenvironment and the interplay between precancerous cells and various stromal components (e.g., immune cells, fibroblasts, adipocytes, endothelial cells). Further facilitating these objectives, organoid co-culture systems are being rapidly developed and initial successes have been reported on fibroblast and T cells [72]. Relatedly, co-culture of BE organoids with neutrophils has also been established to characterize the inflammatory response during BE-associated neoplastic evolution [73].

In summary, organoid modeling is an advanced *in vitro* system faithfully preserving physiological and pathological characteristics

of the modeled tissue, such as self-renewal, multilineage differentiation, signaling nodes and histopathology. These distinctive advantages place organoid modeling technology in a unique position for the investigation of the early neoplastic transformation and premalignant biology. Moving forward, the application of single-cell genomic methodologies such as single-cell Perturb-Seq and other single-cell genome-wide genomic screens in precancerous organoids holds the potential to identify novel biomarkers and actionable targets for early cancer detection, intervention and prevention [74].

CRediT authorship contribution statement

Hua Zhao: Conceptualization, Investigation, Writing – original draft, Visualization. **Casey Collet:** Conceptualization, Writing – original draft, Writing – review & editing. **Dongzi Peng:** Investigation, Writing – review & editing. **Uttam K. Sinha:** Conceptualization, Resources, Funding acquisition. **De-Chen Lin:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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