Running title: Wnt and patient-derived organoids for personalized medicine

The central role of Wnt Signaling and Organoid Technology in Personalizing anti-cancer therapy.

Elizabeth Vincan[1,2#], Renate HM Schwab[1], Dustin J Flanagan[1], Jean M Moselen[1], Bang Manh Tran[1], Nick Barker[4], Toby J Phesse[1,3].

[1]University of Melbourne & Victorian Infectious Diseases Reference Laboratory, Doherty Institute of Infection and Immunity, Melbourne, VIC 3000, Australia

[2]School of Biomedical Sciences, Curtin University, Perth, WA 6845, Australia

[3]European Cancer Stem Cell Research Institute, Cardiff University, Cardiff CF24 4HQ, UK

[4]Institute of Medical Biology, Singapore 138648, Singapore

#Corresponding author:

Prof Elizabeth Vincan

Victorian Infectious Diseases Reference Laboratory,

Doherty Institute of Infection and Immunity,

Melbourne,

VIC 3000, Australia

evincan@unimelb.edu.au

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Abstract

The Wnt pathway is at the heart of organoid technology, which is set to revolutionize the cancer field. We can now predetermine a patient's response to any given anti-cancer therapy by exposing tumor organoids established from the patient's own tumor. This cutting-edge biomedical platform translates to patients being treated with the correct drug at the correct dose from the outset, a truly personalized and precise medical approach. A high throughput drug screen on organoids also allows drugs to be tested in limitless combinations. More recently, the tumor cells that are resistant to the therapy given to a patient were selected in culture using the patient's organoids. The resistant tumor organoids were then screened empirically to identify drugs that will kill the resistant cells. This information allows diagnosis in real-time to either prevent tumor recurrence or effectively treat the recurring tumor. Furthermore, the ability to culture stem cell-derived epithelium as organoids has enabled us to begin to understand how a stem cell becomes a cancer cell or to pin-point the genetic alteration that underlies a given genetic syndrome. Here we summarize these advances and the central role of Wnt signaling, and identify the next challenges for organoid technology.

1. Introduction

Stem cell derived three-dimensional (3D) replicas of organs grown in tissue culture, termed organoids, have led to remarkable advances in stem cell and developmental biology, human disease and regenerative medicine[1]. "Organoid" is a term originally used by developmental biologists working with tissue explants to unravel the mechanisms of organogenesis. The term literally means "organ-like". More recent use of the term organoid is defined as a 3D structure established from stem cells and consisting of organ-specific cell types that self-organize to mimic their tissue of origin[2,3]. Organoids can be initiated from two main types of stem cells (summarized in Table I). The first stem cell type is the pluripotent embryonic stem (ES) cell or the induced pluripotent stem cell (iPS). For iPS, adult cells are artificially reprogrammed to pluripotency[4], and then differentiated towards different organ cell types using cues that have been identified to orchestrate the development of those organs during embryogenesis and organogenesis[3,5]. Diverse tissue and organ cell types can be derived from a pluripotent stem cell.

[**Table I** here, vertically]

By contrast, the second type of stem cells, the tissue restricted adult stem cells, have a "memory" of their tissue of origin and self-organize and differentiate into structures that contain the different tissue-specific cell types; they recapitulate the characteristics of tissue function and architecture[1]. The culture conditions that were developed to establish adult tissue stem cell-derived organoids were then adapted to growing organoids from diseased tissues such as cancers (Table I). This innovation has led to one of the most important advances in cancer research – high throughput drug prescreening, in a clinically relevant time frame, on patient-derived tumor organoids to personalize treatment[6,7]. In this chapter we briefly summarize the discoveries that led to adult stem-cell-derived organoid technology, the central role of Wnt signaling in this advance and how this advance is poised to revolutionize anti-cancer treatment. We also highlight the next challenges for

patient-derived tumor organoid technology in the quest for curative anti-cancer treatment. Improved survival is clearly a great outcome for anti-cancer treatment, but the ultimate goal is curative treatment where the tumor cells are eliminated.

2. Wnt signaling pathway

The Wnt signal transduction pathway has several branches that are β-catenin-dependent and β-catenin-independent (i.e. the calcium and planar cell polarity pathways) and the core components of these are highly conserved through evolution[8,9]. Here we give a brief overview of the Wnt/β-catenin branch[10] as it is critical to stem cell function and organoid formation. In the absence of a Wnt ligand, β-catenin is primarily engaged in cell-cell adherens junctions. Any free newly synthesized β-catenin is rapidly recruited to a cytoplasmic destruction complex that contains several proteins including adenomatous polyposis coli (APC), Axin and two kinases, casein kinase-1 (CK1) and glycogen synthase kinase-3 (GSK-3) that phosphorylate the recruited β-catenin. Subsequently, phosphorylated β-catenin is ubiquitylated by β-transduction repeat-containing protein (β-TrCP) targeting it for proteasomal degradation (**Figure 1A**). Upon Wnt binding to Frizzled (Fz) and its co-receptor LRP (low-density lipoprotein-related protein), the intracellular domain of LRP is phosphorylated and the destruction complex relocates to the receptor complex to transduce the signal. The mechanisms are still unclear, but ubiquitylation of β-catenin is inhibited and β-catenin escapes degradation. It accumulates in the cytoplasm and eventually translocates to the nucleus where it forms a transcriptionally active complex with T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors. β-catenin-mediated activation of transcription replaces Groucho (or TLE) – mediated repression of TCF/LEF to initiate the expression of Wnt/β-catenin target genes (**Figure 1**B)[11].

The Wnt/β-catenin target genes that are induced are context-dependent and can mediate diverse outcomes in different cell types, even if the cell types are within the same tissue. For example, in the intestinal epithelium, active TCF/β-catenin transcription leads to the differentiation of Paneth cells[12] but it is also necessary for the proliferation of stem cells and early progenitors[13], thus highlighting distinct target gene repertoires within the same tissue[14]. An emerging theme in the field is that it is the fold change in the level of nuclear β-catenin rather than the absolute amount of β-catenin in the nucleus dictating activation of Wnt signaling[15]. Also, the pathway is not as simple as an "ON-OFF" switch; the level of Wnt activation dictates the ultimate cellular outcome. The latter led to the proposal of the "just right" or Goldilocks model of Wnt signaling[16] with extensive regulation of the signaling cascade at each step of the pathway, especially in cancer cells[8,17].

Indeed, tight regulation of events at the plasma membrane certainly come to the fore in cancer. This is perhaps not surprising given that the tumor cell microenvironment influences cell behavior and the Wnt pathway plays a key role in this regulation. This has been particularly well documented for colon cancer[18,19] (addressed further in section 5 below). Several types of inhibitors of Wnt signaling are *bone fide* tumor suppressors in diverse cancers, including colon cancer[20]. Wnt pathway inhibitors that directly bind Wnt ligands, such as the Frizzled-related proteins (sFRPs) and Wnt inhibitory factor (WIF), can potentially affect any branch of the Wnt pathway. While other inhibitors like the DKK family act specifically on the Wnt/β-catenin branch as they block Wnt binding to LRP. sFRPs[21-23], WIF (Wnt inhibitory factor)[24] and DKK (Dickkopf)[25] are epigentically silenced in colon cancer for example, implicating a role for active Wnt signaling from the receptor complex[20].

[Figure 1 here, horizontally, full page]

3. Organoids derived from adult epithelium

Adult stem cell-derived organoids were first established from the epithelium lining the mouse intestine[26]. Several discoveries led to this game-changing achievement. The first was the demonstration in 1998 by Korinek and colleagues that Wnt signaling is critical for intestinal stem cells. Deletion of the gene that codes for Tcf4, the downstream effector of Wnt signaling (**Figure 1**), from the developing mouse intestine led to a depletion of the putative epithelial stem cell compartment and post-natal death[13]. A similar depletion of the stem cell compartment was observed when other components of the Wnt pathway, for example β-catenin[27], were depleted from the epithelium or the Wnt-inhibitor Dkk-1 was overexpressed[28,29].

The next important discovery almost a decade later was identifying Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5), a highly expressed Wnt target gene in the intestinal crypt progenitor compartment[30], as an exclusive marker of adult intestinal stem cells[14]. The epithelial lining of the intestine has the fastest turnover of any tissue in the adult, with the entire lining being replaced every several days. Using mice engineered to track Lgr5 positive ($Lgr5^+$) stem cells and their progeny led to the demonstration that this turnover of the epithelium is indeed maintained by $Lgr5^+$ stem cells that reside at the base of invaginations within the epithelium called the crypts of Lieberkuhn[14] (**Figure 2A**).

The third important advance was the demonstration that, given the correct growth factors and environment, a single $Lgr5^+$ stem cell can self-renew and give rise to daughter cells that self-assemble to form a complex three dimensional (3D) structure containing all known intestinal epithelial cell types, thus forming ever-expanding 3D intestinal organoids in tissue culture[26] (represented in **Figure 2D**). To demonstrate this, $Lgr5^+$ intestinal stem cells were purified using Lgr5-promoter driven EGFP expression and FACS sorting. The ability to grow “mini-guts” in tissue culture has enabled the identification and characterization of the stem cell niche factors[31,32],

and an understanding of the molecular mechanisms of Wnt signaling in stem cell maintenance, epithelial cell differentiation[33] and aberrant Wnt signaling at the initiation of cancer[34].

[**Figure 2** here, horizontally, full page]

Lgr5 stem cells generate 3D organoids

To establish intestinal organoids, the crypts of the epithelium (**Figure 2A, B**) are isolated and re-suspended in a solubilized basement membrane preparation rich in extracellular matrix proteins (called Matrigel), which is liquid at 4°C but sets as a gel at 37°C. This provides the 3D matrix for the crypts to form organoids. Once the gel has set, it is overlaid with medium containing growth factors that recapitulate the *in vivo* crypt niche. Just three factors are necessary to maintain ever-expanding intestinal organoids: R-spondin to potentiate endogenous Wnt signals; the BMP inhibitor Noggin and epidermal growth factor (EGF)[26]. Within 24 hr of plating, the crypts form cysts that are already polarized with stem cells to one pole (**Figure 2C**); after several days in culture, organoids are formed with defined crypt and villus domains (**Figure 2D**); the different epithelial cell types are represented in roughly equivalent relative proportions to the normal epithelium[26]. The organoids shed dead cells into the lumen and will eventually burst open, releasing dead cells, if not passaged. The intestinal organoids continue expanding and require fresh growth factors every other day, and passaging each week[26]. The organoids are genetically stable with continuous passage[35], can be manipulated genetically using current genetic tools such as CRISPR/Cas9 genetic editing[36,37], Cre-LoxP mediated gene manipulation[38] or transfection/transduction[39]. Intriguingly, when embedded within contracting collagen gels, intestinal organoids fuse to form macroscopic intestinal tubes that have a continuous lumen lined by villus cell types and crypt-like structures budding from the tube into the collagen[40]. Thus, lengths of

intestine can be generated in tissue culture without the need for complex tissue-engineering scaffolds.

Organoids also hold great promise for regenerative medicine and transplantation, but one drawback is the cost of recombinant growth factors and animal cell line derived matrix (Matrigel is derived from mouse sarcoma cell line). The cost of reagents was partly alleviated using conditioned media from cell lines that secrete the growth factors (e.g. Wnt3a, R-spondin, noggin producing cell lines[41]). However, conditioned medium is not defined as it contains unknown components and thus is not applicable for generating tissue for transplantation and other clinical applications. To this end, several avenues are being investigated to overcome these barriers. For example, stabilizing Wnt in serum free medium. Unlike intestinal organoids, several other organoid types require Wnt in the growth factor cocktail. However, Wnt ligands are hydrophobic and require serum for optimal activity; recombinant Wnts perform poorly compared to conditioned medium. A glycoprotein called afamin was recently identified as a component of serum that stabilizes Wnt and purified afamin improves the performance of recombinant Wnt[42]. Other groups have used phospholipids and cholesterol carriers to stabilize Wnt[43] or water soluble surrogate Wnt agonists that activate Wnt/β-catenin signaling[44]. Also, advances in bioengineering[45] has seen the development of modular synthetic hydrogel matrices that replace the need for Matrigel. Extracellular proteins were incorporated into the hydrogel networks and the components necessary for organoid formation, stem cell maintenance and cell differentiation have been defined[46]. Furthermore, these synthetic matrices allow the “stiffness” of the matrix to be varied, as the physical properties of the cellular microenvironment also affects cell behavior[46]. This is reminiscent of the well-documented effects of matrix “stiffness” on cancer cell behavior[47].

4. Organoids derived from patient tumors

Once the culture techniques for growing intestinal organoids were established, variations on

the same culture protocol led to the establishment of organoids from several other gastrointestinal tissues[41,48] as well as many other stem-cell maintained adult tissues (several comprehensive recent reviews[49-51]). Clevers and colleagues also adapted the organoid protocols to grow patient-derived colon cancer tumor organoids. The mini-tumor organoids similarly recapitulate the features of actively growing colon cancers and can accurately predict the patient's response to treatment[6]. Consequently, mini-gut and mini-tumor organoid platforms provide powerful tools for drug discovery and predictive drug-response diagnostics for cancer treatment. Generally, about 60% of colon cancer patients respond to any particular therapeutic regimen. The non-responders are then treated with alternative drug regimens. The ability to pre-screen the drugs on an individual patient's tumor cells, both singly and in limitless combinations with other drugs, means that the patient is treated with the drug regimen that will work on their tumor from the outset[6]. This not only saves time between diagnosis and effective treatment but also eliminates unnecessary treatment and the consequent side effects. Here we focus on colon cancer to highlight the advances and the next challenges of organoid technology in the cancer field.

Wnt signaling in colon cancer

Colon cancer starts in the simple epithelium that lines the colon. Most colon cancers in humans, including somatic cancers, arise from adenomas (non-cancerous tumors or polyps) that harbor truncating mutations in the *Adenomatous Polyposis Coli (APC)* tumor suppressor gene[52,53]. These mutations in the *APC* gene lead to constitutive activation of the Wnt/β-catenin pathway[54,55] and the formation of adenomas in the epithelium of the colon. The adenomas can then progress to cancerous tumors through the accumulation of mutations to activate oncogenes and inactivate tumor suppressors[56]. Human colon cancers that do not harbor *APC* gene mutations often have oncogenic mutations in the β-catenin gene (*CTNNB1*)[53]. Thus, the vast majority of colon cancers have mutations in the intracellular pathway components that activate Wnt/β-catenin signaling.

APC facilitates the phosphorylation and subsequent targeting of β-catenin for proteasomal degradation (**Figure 1**). In colon cancer, mutations to the *APC* gene lead to a truncated APC protein and this facilitative function is lost, and consequently, the constitutive activation of the pathway[54,55]. Oncogenic mutations to *CTNNB1* alter the negative regulatory domain of β-catenin at the N-terminus, and again, lead to constitutive activation of the Wnt pathway[57]. Transgenic mice harboring these alterations to APC[58,59] or β-catenin[60] genes develop multiple intestinal adenomas with active Wnt signaling.

In addition to these mutations that activate the pathway, Wnt signaling is further regulated through multiple mechanisms in colon cancer[8]. The Frizzled (FZD) receptors and Wnt ligands are over-expressed in colon cancer and can modulate the pathway[61,62], while naturally occurring inhibitors of Wnt-FZD interaction (e.g. sFRP) are epigenetically silenced and are *bona fide* tumor suppressors in human colon cancer [20,21,23]. Curiously, the net effect is to "constrain" the Wnt signaling pathway in the cancer cells to a sub-maximal level of activation as signaling can be decreased[23] and increased experimentally[63] and is hyper-activated in cancer cells engaged in tumor invasion[64,65].

Genetic dissection of colon cancer development using organoids

In addition to constitutively active Wnt signaling at the initiation of colon cancer, progression from adenoma to carcinoma requires mutations in genes in other oncogenic pathways. The adenoma-carcinoma sequence was originally proposed by Vogelstein[56] and colleagues based on the analysis of mutations present at each stage of tumor progression. Sequential acquisition of mutations that lead to functional loss of function of other tumor suppressors such as *TP53* and *SMAD4*, as well as activating mutations in other oncogenes such as *KRAS*. Intriguingly, the

introduction of these mutations sequentially and in combination into normal epithelial cells using organoids and gene editing (CRISPR/Cas9) has not only identified the minimal mutations necessary for cancer development but has also provided an explanation of the growth factor requirements for organoid growth[66]. That is, each genetic mutation alleviates the need for a growth factor. Mutations to activate Wnt signaling alleviate the need for R-spondin and Wnt, mutation in *KRAS* alleviate the need for EGF and inactivating mutation in *SMAD4* alleviate the need for Noggin to inhibit BMP signaling. With the additional mutation of *TP53*, the quadruple mutant organoids grew without the need for growth factors and formed invasive subcutaneous tumors[66]. Using a similar strategy, Matano and colleagues showed that mutation in PI3K pathway (*PIK3CA*) can substitute for *KRAS* mutation[67]. These initial studies have been expanded upon to demonstrate that quadruple mutant organoids (i.e. *APC, KRAS, p53, SMAD4*) yield invasive tumors in an orthotopic mouse model[68]. These findings indicate that the loss of niche dependency leads to the ability to metastasize to secondary organs, at least in an experimental metastasis model.

Patient-derived tumor organoids for drug pre-screen and Biobanking

An important application of organoid technology has been the ability to establish tumor organoids from resected and biopsy samples and to adapt the mini-tumor organoids to high-throughput drug screens. This was first achieved by the Clevers lab in a retrospective study where patient drug response was compared to the response of the corresponding patient-derived organoids, and coupling this with genomics to identify gene-drug associations[6]. The patient-derived tumor organoids are a faithful replica of the patient's tumor and can be established from primary tumors and metastases[69]. Patient-derived organoids and the "omic" analyses of these (genomic, epigenomic, transcriptomic, proteomic), have revealed that normal organoids are more stable in culture than malignant tissues[70,71], which might have been expected given that chromosomal instability is a common feature of cancer, but needed formal demonstration. Furthermore, by

establishing a biobank of patient-derived tumor and normal tissue organoids, drug discovery and "clinical trials" are expedited. Novel drugs can be tested by simply thawing out a panel of tumor organoids and the high-throughput format allows for multiple combinations, titrations etc. A not-for-profit foundation (HUB) has been established by the Clevers group to advance this technology (see http://hub4organoids.eu/).

Patient-derived xenografts (PDX) have become the gold standard for "personalized anti-cancer treatment" (numerous recent reviews e.g. Byrne and colleagues[72]). However, PDX models are limited in their application to personalized medicine for several seasons. The success rate of establishing xenograft tumors from patient material is low, the time to establish tumor xenografts is slow, and the cost of mouse models are just a few of the caveats. PDX also does not lend itself to high throughput. Delivering information in a clinically relevant timeframe is a real hindrance to using PDX models as a diagnostic tool. Patient-derived organoids fulfil this unmet need for personalizing anti-cancer diagnosis and treatment. Biobanks like the HUB are being established around the world, for example the nonprofit organization ALOA (Australian Living Organoid Alliance).

DasGupta and colleagues recently took the high-throughput screen on patient-derived organoids to a new dimension[73]. They generated a library of patient derived organoids and PDX models from head and neck squamous cell carcinomas (HNSCCs) and used these to select for tumor cells that are resistant to the standard treatment given to the patient. The resistant organoids were then comprehensively interrogated to identify patient-specific gene signatures that could potentially underlie the resistance to therapy. Using this strategy they identified that selection for YAP-1 (Yes-associated protein-1) positive cells paralleled failed therapy; implicating YAP-1 is a putative biomarker for resistance[73]. Tumors are heterogeneous and the ability to select resistant cells using organoid culture coupled with the power of "omics" analyses allows diagnosis in real-time. Such a

high throughput drug screen is not possible with any other patient-derived model. Numerous other cancers and tissues have been effectively modelled using organoids and these have been covered by a number of comprehensive recent reviews[74]. Next we will highlight the new challenges for patient-derived organoids.

5. Modelling dormant tumor cells: the next frontier for tumor organoids

One limitation for mini-tumor organoids as a drug screen is that the ever-expanding tumor organoids mimic the actively growing tumor cells. The mini-tumor organoids do not mimic the dormant tumor cell state. The key to curative cancer treatment is to therapeutically target and eliminate the disseminated dormant tumor cells that eventually re-establish tumors at secondary sites and are ultimately the cause of death. Some therapies will target actively dividing as well as dormant tumor cells but we need to be able to establish "dormant" organoid cells from the patient's mini-tumor organoids to screen for these. This is the next challenge for the organoid platform but we have clues about how to do this from other model systems.

Reversible phenotype transitions underlie metastasis

Although most human colon cancers are relatively well differentiated with an epithelial phenotype, in localized areas, termed the "invasive front", the tumor cells take on a more mesenchymal phenotype that is associated with migratory and invasive properties, and the cells shut-down cell proliferation[18]. This phenotypic change, termed epithelial-to-mesenchymal transition (EMT), is thought to enable the tumor cells to dissociate from the tumor mass and disseminate to other organs in the body. The disseminated tumor cells are dormant and acquire resistance to therapies, particularly therapies that target actively dividing cells such as chemotherapy and radiation therapy. The EMT program also induces stem cell-specific gene expression, thus

promoting self-renewal properties[19]. Dissemination can occur early in the disease process, and tumor cells can sit dormant for many years. However, for the tumor cells to re-instate tumor growth at the secondary site, the cells must undergo the reverse transition, mesenchymal-to-epithelial transition (MET), because the secondary tumors recapitulate the differentiated epithelial phenotype of the primary tumor[18,75].

An in vitro model of tumor morphogenesis

Modelling dormant tumor cells

These reversible phenotype transitions have recently been modelled in a human colon cancer cell line that grows as an organoid sphere in tissue culture[63,76]. The parental cell line LIM1863[77] grows as spheres of epithelial cells that are highly polarized along the baso-lateral axis and are organized around a central lumen. These spheres can spontaneously anchor to the tissue culture plastic and form an adherent monolayer patch. After 3 to 4 days in culture, cells in the monolayer patches re-organize to reform the spheres that eventually float freely in the tissue culture medium and the whole process starts again. The parental cell line was adapted to efficiently undergo this spontaneous, reversible transition between monolayer and organoid sphere and the adapted cell line is called LIM1863-*Mph* (for *m*or*ph*ogenetic)[63] (**Figure 3A**). Immunofluorescence confocal microscopy for the junctional protein ZO-1 clearly shows the transition between monolayer and organoid sphere (**Figure 3B**)[78]. Molecular and phenotypic analysis of the cells during these transitions revealed that the features of EMT and MET that underscore colon cancer metastasis are faithfully recapitulated in this model system[63,76,78,79]. Importantly, the monolayer cells (EMT state) decrease cell proliferation and are resistant to agents that block cell proliferation (e.g. Mitomycin C)[63] and thus mimic the properties of chemo resistant mesenchymal invasive front cells. The epithelial spheres can be induced to undergo EMT with TGFβ and TNFα treatment; however, this

transition is not reversible[76]. Nonetheless cytokine-induced, and spontaneous, monolayer formation and the reverse transition in the LIM1983-*Mph* cells provide clues about the underlying mechanisms of dormancy which could be adopted for the tumor mini-organoid platforms. Notably, the monolayer cells are resistant to the PI3K inhibitor LY49002. As noted above, PI3K is one of the genetic insults that converts normal cells to cancer cells in an organoid model[67], yet the LIM1863-*Mph* cells are resistant to a PI3K inhibitor when in the mesenchymal state even though they are sensitive to it in the epithelial state[63].

[**Figure 3** here, vertically, half page]

The LIM1863-*Mph* tumor morphogenesis model highlights one caveat for patient-derived tumor organoids – modeling reversible tumor dormancy. Indeed, studies to date reveal that metastasis relies on subtle changes rather than "driver" gene mutations. For example, in one study mutant organoids engineered from human normal epithelium to carry the driver mutations seen in colon cancer formed micrometastases when injected into the spleen of mice but failed to colonize the liver, the usual metastatic site for colon cancer. In contrast, mutant organoids derived from human adenomas formed liver metastases when the same drivers were introduced[67]. Similarly, tumor organoids derived from colon metastases metastasize better than their matched primary tumor, despite having indistinguishable genetic mutations and niche requirements[7]. Consequently, human genomics needs to be coupled to epigenomics and phenomics if we are to unravel the mechanisms of tumor dormancy and mechanisms of metastasis.

Acute high Wnt/β-catenin signaling in MET

Another intriguing feature of the LIM1863-*Mph* is the dynamic regulation of the levels of nuclear β-catenin, the hallmark of active Wnt/β-catenin signaling (**Figure 1B**). The organoid cells

and the monolayer cells have very low but detectable levels of nuclear β-catenin[63]. This is not surprising as the LIM1863 cells harbor truncating mutations in the *APC* gene[80]. However, as the monolayer cells start to transition back to epithelial morphology and start to lift off the tissue culture plastic and re-organize themselves into spheres, there is a dramatic transient increase in nuclear β-catenin, which is concomitant with a sharp increase in cell division (Ki-67 staining)[63]. Cells in the organoid spheres continue to divide (maintain strong Ki-67 staining) but the level of nuclear β-catenin decreases to just detectable levels. As expected, the organoid sphere cells are susceptible to agents that target cell proliferation (Mitomycin C)[63] and the PI3K inhibitor LY49002 (data not shown). Collectively, this indicates that emergence from a mesenchymal monolayer ("dormant") state (i.e. MET) requires a sharp burst of Wnt/β-catenin signaling[63]. This was confirmed recently by an independent study that showed lithium chloride (LiCl), a known activator of Wnt/β-catenin signaling[63,81], induced MET in primary colon cancer cell cultures[82].

This requirement for Wnt/β-catenin signaling for MET provides several novel avenues to combat the formation of metastases. Experimentally it was shown that Wnt is necessary for MET in the LIM1863-*Mph* cells[78] and the Wnt receptor Frizzled-7 was identified as the necessary Wnt receptor[63]; thus therapeutic targeting of Frizzled-7 would target both actively dividing and dormant tumor cells. Indeed, inhibition of Frizzled-7 mediated signaling in colon cancer cells potently blocked colon cancer xenograft growth[83].

Another avenue might be to activate Wnt/β-catenin signaling in the dormant tumor cells to induce MET and render them susceptible to conventional chemotherapy and radiotherapy. Activating Wnt signaling may sound risky, but lithium could potentially serve this purpose. Lithium is an FDA-approved and preferred drug for the treatment of mood disorders, and evidence is emerging about its potential use as an anti-cancer drug in colon[84-86] and other cancers[87]. Importantly, there is no increased risk, in fact a slight decrease, of cancer in psychiatric patients

treated with lithium[88]. Re-purposing lithium to activate the Wnt/β-catenin pathway immediately before administering conventional chemotherapy and radiotherapy might have a beneficial effect in colon cancer.

6. Conclusions

In this chapter we have tried to highlight the current state of the tumor organoid field, which is advancing at an astronomical rate. Mini-gut and mini-tumor organoids have revolutionized our understanding of the molecular mechanisms that underlie transition from normal epithelial stem cell to cancer cell, identify the molecular drivers of cancer cells and predict their susceptibility to anti-cancer drugs. The ability to conduct drug screens on dormant patient tumor cells is the next frontier and vital to our quest to improve cancer patient survival rates towards a cure.

Indeed, we propose that the most important challenge for organoid technology and high throughput drug screens is to model patient-derived dormant tumor cells, because the barrier to curative treatment is metastasis, the cause of death in most cases. Tumor cells can spread to other organs early in the disease, long before diagnosis, and can lay dormant in the secondary organs for years[89]. Organoids with dormant tumor cells have the potential to identify therapies to eliminate these cells. We have clues from model systems such as LIM1863-*Mph* described above but also possibly from $Lgr5^+$ stem cells. It was recently shown that $Lgr5^+$ stem cells can be made quiescent *in vitro,* and that the quiescent state is reversible[90]. We already know that $Lgr5^+$ colon cancer cells maintain tumor growth and progression[91,92], akin to the role of $Lgr5^+$ intestinal stem cells maintaining the intestinal epithelium. Thus, $Lgr5^+$ cancer cells behave like cancer stem cells. Notably, cancer stem cell plasticity, where Lgr5 negative cells can revert to being $Lgr5^+$, has also been recently demonstrated in a mouse model[92], again reminiscent of intestinal stem cell plasticity where daughter cells can revert to the $Lgr5^+$ state to repopulate the crypt[93-95]. Perhaps the $Lgr5^+$ cancer cells will also provide the clues to tumor dormancy.

In 1960s Gurdon demonstrated that an adult nucleus can be the blueprint for an organism[96], and the eventual adoption of this knowledge in 2006 to induce pluripotency in adult cells by Yamanaka and colleagues[4], and the ability to derive organoids from these pluripotent stem cells, has led to a steady stream of organoid-based publications. However, the demonstration in 2009 that adult stem cells can be coerced to generate their tissue of origin *in vitro* has led to an exponential increase in organoid-based publications[1]. This explosion in knowledge is set to be fueled by combining the two varieties of stem cell-derived organoids (**Table I**). Adult tissue stem cell-derived organoids do not contain other cell types in the organ e.g. no immune cells, neuronal cells or stromal cells for example. However, by adding patient iPS-derived cell types to patient adult stem cell-derived organoids, we can start to build more complex tissues or even organs, because autologous iPS cells can provide cells types that are present in the organ but are not derived from the adult tissue-restricted stem cell. For example, recently iPS and adult stem cell derived organoids have been combined to generate stem-cell derived intestinal tissue with a functional enteric nervous system[97]. This is just the beginning - very exciting times ahead.

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