



# Bladder organoids: a step towards personalised cancer therapy?

Sreemoti Banerjee, Jennifer Southgate

Jack Birch Unit for Molecular Carcinogenesis, Department of Biology, York Biomedical Research Institute, University of York, York, UK

*Correspondence to:* Jennifer Southgate. Jack Birch Unit for Molecular Carcinogenesis, Department of Biology, York Biomedical Research Institute, University of York, York YO10 5DD, UK. Email: j.southgate@york.ac.uk.

*Provenance:* This is an invited article commissioned by Section Editor Xiao Li (Department of Urology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & Nanjing Medical University Affiliated Cancer Hospital, Nanjing, China).

*Comment on:* Mullenders J, de Jongh E, Brousal A, *et al.* Mouse and human urothelial cancer organoids: A tool for bladder cancer research. *Proc Natl Acad Sci U S A* 2019. [Epub ahead of print].

Submitted May 24, 2019. Accepted for publication Jun 11, 2019.

doi: 10.21037/tau.2019.06.10

**View this article at:** <http://dx.doi.org/10.21037/tau.2019.06.10>

Urothelial bladder cancer (UBC) is complicated by its underlying genetic diversity that renders prognosis and treatment a challenge and imposes a significant health and economic burden. Although the non-muscle invasive form of bladder cancer (NMIBC) is responsive to treatment, a significant proportion of tumors recur and patients require lifelong monitoring to identify cancers that become muscle-invasive. Most patients with muscle-invasive bladder cancer (MIBC) present late and show poor response to therapy, with attendant poor survival. This underpins the clinical need for progress in bladder cancer research and translation.

A longstanding and relatively simple tool to study bladder cancer comes in the form of established human bladder cancer-derived cell lines (1,2). Grown in two-dimensional adherent cultures, typically in medium supplemented with serum, these cell lines display complex karyotypes that reflect the genesis and evolution of the cancer, but also selection pressures imposed during adaptation to survival *in vitro*. Although frequently criticised, established human UBC cell lines do provide an invaluable expandable and experimentally-tractable research resource, reflecting a spectrum of the genetic changes captured in large scale sequencing of primary tumors. Said cell lines also retain the capacity to recapitulate more complex phenotypes and originating invasive behaviours when combined into more complex heterotypic (3), 3D tissue-engineered (4) or *in vivo* systems (1,5).

One of the limitations of UBC cell lines has been the small proportion of primary bladder tumors that will establish in culture, meaning that there is an inherent bias

towards generating UBC lines from tumors with particular characteristics suited for growth *in vitro*. This contrasts with normal human urothelium that can be routinely established in serum- and stromal-free primary culture to generate finite cell lines (6), indicating that the inherent plasticity and regenerative characteristics of normal human urothelium becomes more restricted following neoplastic transformation.

Arising in part from the large-scale transcriptomic stratification and sub-typing of UBC has been the idea of selecting therapies tailored to an individual's tumor. This is incumbent both on developing appropriate screening tools and on improving the efficiency of establishing individual patient-derived cancers for testing *in vitro/ex vivo*. Not only this, but incorporation of stromal and immune components may be seen as critical for developing informative, predictive systems for delivering effective tailored therapies. This is both ambitious and technically challenging.

A relatively recent approach has been the development of organoid systems. These have been applied to a number of different cancer types [reviewed (7)] including prostate (8,9), colorectal (10-13), gastric (14,15), pancreatic (16,17), liver (18) and breast (19,20). Organoids represent collections of cells harvested from a particular organ that are grown in specified 3D environments on extracellular matrix proteins using optimised cocktails of growth factors to recapitulate many aspects of the tissue of origin. Several recent publications have described the development of bladder organoids (21-23). Lee *et al.* demonstrated that the mutational profiles of the bladder cancer-derived organoids

were highly consistent with their parental tumors and reflected common genomic alterations in human UBC (21). Moreover, organoids maintained *in vitro*, as orthotopic xenografts *in vivo*, or as xenograft-derived organoids *in vitro* all displayed clonal evolution (21), a process observed during UBC progression *in vivo* (24). Yoshida *et al.* nicely illustrated the greater potential of 3D-organoids over 2D adherent cell lines by demonstrating differential activity of the Wnt/ $\beta$ -catenin pathway in driving proliferation in 3D systems (23). This is an important consideration in developing the most appropriate platform for individual drug screening.

Mullenders *et al.* have recently described a bladder organoid culture system applied to normal mouse urothelium and to a series of 53 primary human UBC (22). The system relies on an optimised growth medium to support the long-term sustained growth of organised 3D structures, which can be maintained, expanded and sub-cultured *in vitro*. The normal mouse urothelial organoids provide a complementary research tool to the more idiospecific human primary UBC. As described (22), the mouse urothelial organoids were genetically-stable over 60 passages and tractable to manipulation of differentiation and genome. The full potential of this organoid system to recapitulate tumorigenic changes is at present unclear given a lack of any apparent phenotype in organoids constituted with edited urothelial cells carrying *p53/Stag2* double knockouts.

Across the bank of UBC organoids, Mullenders and colleagues describe a diversity of morphological and histological characteristics, including evidence of intra-tumoral heterogeneity from the differential expression of CK5 and CK20. Nevertheless, a major gap in understanding the urothelial organoids remains as the authors do not appear to have considered or characterised the existence of stromal cells in either mouse- or human bladder cancer-derived organoids. As the entire tissue is reported to be disaggregated into the single cell suspensions used to constitute the organoids, it is reasonable to expect that the organoids will be composed of both urothelial and stromal-derived cells. The presence of stromal cells would have strengthened the justification of the authors for using these organoids as a more histopathologically-relevant model system for studying bladder cancer. By comparison, other authors have attempted to recapitulate the bladder cancer environment by combining UBC cell lines with stroma in “tissue-engineered” 3D bladder cancer models (3,4).

Mullenders and colleagues indicate that human urothelial

organoids derived from MIBC patients undergoing radical cystectomy could be established with at least 50% efficiency. While for most patients the organoids were initiated from bladder tumor and macroscopically normal urothelium, there was no clear indication of the efficiency of the organoid cultures from normal versus cancer regions. A possible explanation for the failure of some MIBC organoids could be the high genetic instability of the cancer cells leading to increased rates of apoptosis in cancer-derived organoid cultures, as has been hypothesized (25).

The Mullenders paper adds to a small but expanding literature that organoids can be established from human bladder cancer tissues to provide an *ex vivo* platform to relate phenotype and/or drug susceptibility to cancer genotype/phenotype through systematic (21) or focused (FGFR3) (22) mutational studies. The authors illustrate the differential response of a few organoid lines to a panel of known chemotherapy drugs, which begins to test the potential for personalised cancer therapies. What will be interesting is to link responses from these organoids with the treatment response of the patients from which they were derived. While these organoid models offer the promise of testing novel cancer therapeutics, there remains a pressing need for validation.

Whilst the long-term goal may be to apply the organoid model to test individual patient response to therapeutics, the organoid system offers other opportunity for basic urothelial cell biology research, not least into the nature of stem cells and regulation of differentiation/growth pathways in normal and neoplastic urothelium. An altered tissue environment is heavily implicated in the development and progression of UBC and the opportunity perhaps also exists to investigate interactive stromal:epithelial signalling pathways such as Wnt, Shh and Notch in matched normal versus tumor organoids.

## Acknowledgments

None.

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved.

## References

1. Masters JR, Hepburn PJ, Walker L, et al. Tissue culture model of transitional cell carcinoma: characterization of twenty-two human urothelial cell lines. *Cancer Res* 1986;46:3630-6.
2. Earl J, Rico D, Carrillo-de-Santa-Pau E, et al. The UBC-40 Urothelial Bladder Cancer cell line index: a genomic resource for functional studies. *BMC genomics* 2015;16:403.
3. Booth C, Harnden P, Trejdosiowicz LK, et al. Stromal and vascular invasion in an human in vitro bladder cancer model. *Lab Invest* 1997;76:843-57.
4. Ringuette Goulet C, Bernard G, Chabaud S, et al. Tissue-engineered human 3D model of bladder cancer for invasion study and drug discovery. *Biomaterials* 2017;145:233-41.
5. John BA, Said N. Insights from animal models of bladder cancer: recent advances, challenges, and opportunities. *Oncotarget* 2017;8:57766-81.
6. Baker SC, Shabir S, Southgate J. Biomimetic urothelial tissue models for the in vitro evaluation of barrier physiology and bladder drug efficacy. *Mol Pharm* 2014;11:1964-70.
7. Xu H, Lyu X, Yi M, et al. Organoid technology and applications in cancer research. *J Hematol Oncol* 2018;11:116.
8. Gao D, Vela I, Sboner A, et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014;159:176-87.
9. Puca L, Bareja R, Prandi D, et al. Patient derived organoids to model rare prostate cancer phenotypes. *Nat Commun* 2018;9:2404.
10. Fujii M, Shimokawa M, Date S, et al. A Colorectal Tumor Organoid Library Demonstrates Progressive Loss of Niche Factor Requirements during Tumorigenesis. *Cell Stem Cell* 2016;18:827-38.
11. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;141:1762-72.
12. van de Wetering M, Francies HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015;161:933-45.
13. Weeber F, van de Wetering M, Hoogstraat M, et al. Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc Natl Acad Sci U S A* 2015;112:13308-11.
14. Seidlitz T, Merker SR, Rothe A, et al. Human gastric cancer modelling using organoids. *Gut* 2019;68:207-17.
15. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018;359:920-6.
16. Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 2015;160:324-38.
17. Seino T, Kawasaki S, Shimokawa M, et al. Human Pancreatic Tumor Organoids Reveal Loss of Stem Cell Niche Factor Dependence during Disease Progression. *Cell Stem Cell* 2018;22:454-67.e6.
18. Broutier L, Mastrogianni G, Verstegen MM, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med* 2017;23:1424-35.
19. Ranftl RE, Calvo F. Analysis of Breast Cancer Cell Invasion Using an Organotypic Culture System. *Methods Mol Biol* 2017;1612:199-212.
20. Sachs N, de Ligt J, Kopper O, et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* 2018;172:373-86.e10.
21. Lee SH, Hu W, Matulay JT, et al. Tumor Evolution and Drug Response in Patient-Derived Organoid Models of Bladder Cancer. *Cell* 2018;173:515-28.e17.
22. Mullenders J, de Jongh E, Brousal A, et al. Mouse and human urothelial cancer organoids: A tool for bladder cancer research. *Proc Natl Acad Sci U S A* 2019;116:4567-74.
23. Yoshida T, Sopko NA, Kates M, et al. Three-dimensional organoid culture reveals involvement of Wnt/ $\beta$ -catenin pathway in proliferation of bladder cancer cells. *Oncotarget* 2018;9:11060-70.
24. Lamy P, Nordentoft I, Birkenkamp-Demtröder K, et al. Paired Exome Analysis Reveals Clonal Evolution and Potential Therapeutic Targets in Urothelial Carcinoma. *Cancer Res* 2016;76:5894-906.
25. Karthaus WR, Iaquinta PJ, Drost J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 2014;159:163-75.

**Cite this article as:** Banerjee S, Southgate J. Bladder organoids: a step towards personalised cancer therapy? *Transl Androl Urol* 2019;8(Suppl 3):S300-S302. doi: 10.21037/tau.2019.06.10