NOVEL THREE-DIMENSIONAL ORGANOID CULTURE REVEALS INVOLVEMENT OF WNT/BETA-CATENIN PATHWAY IN PROLIFERATION OF BLADDER CANCER CELLS

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Introduction

There has been increasing awareness of the importance of three-dimensional culture of cancer cells. Tumor cells growing as multicellular spheroids (organoids) are believed to more closely mimic solid tumors in situ. Meanwhile, Wnt/β-catenin pathway was reported to be upregulated in human bladder cancer specimens. However, no clear evidence has been reported that the pathway is directly involved in proliferation of bladder cancer cells.

In this study, we assessed the involvement of Wnt/β-catenin pathway in proliferation of bladder cancer cells in organoid culture using both immortalized cell lines and primary cells derived from patient samples.

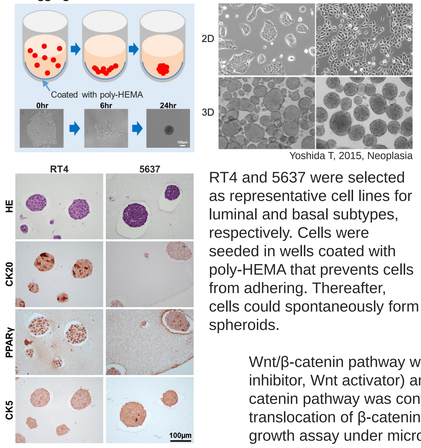
Conclusions

We showed for the first time that Wnt/β-catenin pathway was directly involved in proliferation of bladder cancer cells, suggesting that Wnt/β-catenin pathway may be a potential target for the treatment of a subset of bladder cancer.

The above finding could not be observed when the same cells were grown in conventional two-dimensional adherent cultures, providing a concrete example of why organoid culture is important for cancer research.

Methods

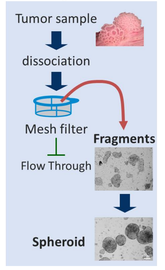
ORGANOID from cell line

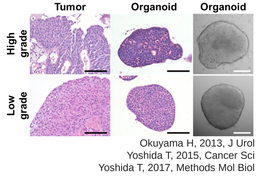


Yoshida T, 2015, Neoplasia

RT4 and 5637 were selected as representative cell lines for luminal and basal subtypes, respectively. Cells were seeded in wells coated with poly-HEMA that prevents cells from adhering. Thereafter, cells could spontaneously form spheroids.

ORGANOID from patient sample





Okuyama H, 2013, J Urol
Yoshida T, 2015, Cancer Sci
Yoshida T, 2017, Methods Mol Biol

Fresh samples were enzymatically dissociated into fragments. Thereafter, fragments were collected and cultured, and were able to spontaneously form spheroids. These spheroids consist of highly purified cancer cells.

Wnt/β-catenin pathway was activated by using a small molecule CHIR99021 (GSK3 inhibitor, Wnt activator) and inhibited by siRNA against β-catenin. Activation of Wnt/β-catenin pathway was confirmed by upregulated AXIN2 mRNA expression and translocation of β-catenin into nucleus. Proliferation of cancer cells were evaluated by growth assay under microscope and ATP viability assay. Differentiation status of organoids over growth was characterized by qRT-PCR and western blot.

Source of Funding

The Greenberg Bladder Cancer Institute Research Grant and the Urology Care Foundation

Results

Figure 1. CHIR inhibited proliferation of cell lines in conventional adherent culture, but promotes proliferation in 3D organoid culture.

(A) In adherent culture, CHIR exhibited an inhibitory effect on proliferation of RT4 and 5637 in a dose-dependent manner. (B-D) However, CHIR promoted proliferation of RT4 and 5637 in organoid culture. Representative images of RT4- and 5637-derived organoids treated with DMSO or CHIR are shown in (B). Growth of organoids was quantified by area of organoids (C) and ATP value (D).

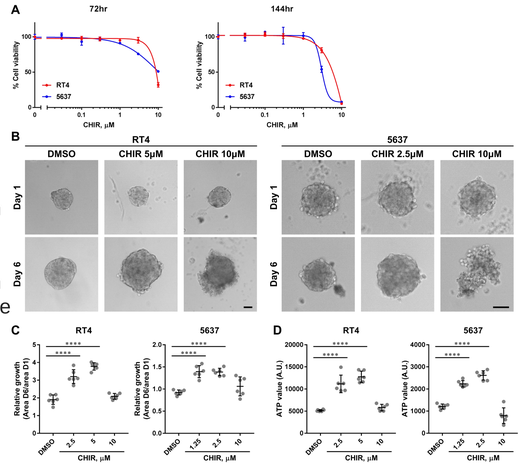


Figure 2. CHIR activates Wnt/β-catenin pathway in bladder cancer cell line-derived organoids.

(A) qRT-PCR showed upregulation of AXIN2 transcripts in CHIR-treated organoids compared to DMSO-treated ones. (B) Western blot analysis demonstrated increased amount of β-catenin protein in CHIR-treated organoids in both cytosol and nuclei.

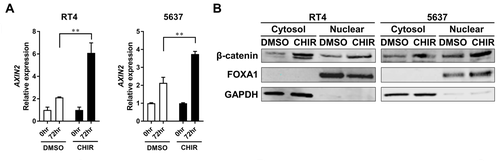


Figure 3. CHIR promotes proliferation of cancer cells in patient-derived organoids with activation of Wnt/β-catenin pathway.

(A-C) Organoids from 4 patient samples were challenged by CHIR treatment. Representative images of patient-derived organoids treated with DMSO or CHIR are shown in (A). Growth of organoids was quantified by area of organoids (B) and ATP value (C). (D,E) qRT-PCR showed upregulation of AXIN2 transcripts in CHIR-treated organoids compared to DMSO-treated ones. Results of BCa#04 and 3 pooled samples are shown in (D) and (E), respectively. (F) Immunocytochemistry demonstrated nuclear localization of β-catenin in CHIR-treated organoids.

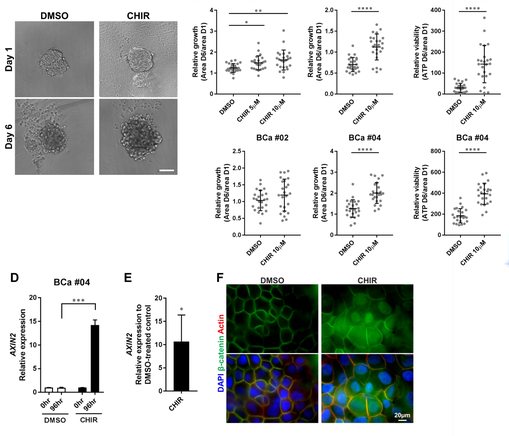


Figure 4. β-catenin is required for growth of bladder cancer organoids.

(A) Western blot shows knockdown efficiency of siRNA against β-catenin in RT4 and 5637. (B,C) After transfection of siCTNNB1, growth of RT4-derived organoids impaired. Representative images were shown in (B), and the growth was quantified and shown in (C). (D,E) After transfection of siCTNNB1, CHIR-inducible growth of 5637-derived organoids was suppressed. Representative images were shown in (D), and the growth was quantified and shown in (E).

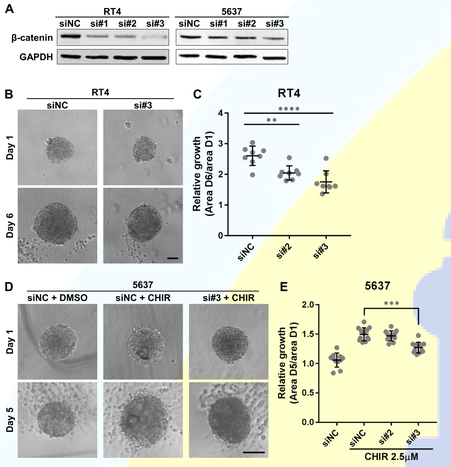
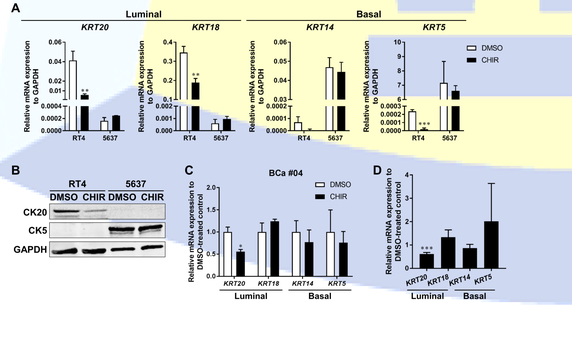


Figure 5. CK20 is less expressed in CHIR-treated bladder cancer organoids.

(A, B) Expression of luminal (CK20, CK18) and basal (CK14, CK5) markers were analyzed by qRT-PCR (A) and western blotting (B) in RT4- and 5637-derived organoids treated with DMSO or CHIR. (C,D) qRT-PCR showed luminal and basal markers expression in CHIR-treated patient-derived organoids compared to DMSO-treated ones. Results of BCa#04 and 3 pooled samples are shown in (C) and (D), respectively. CK20 was consistently less expressed in CHIR-treated organoids.



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