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Platinum Priority – Brief Correspondence – Editor's Choice

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Personalized Drug Sensitivity Screening for Bladder Cancer Using Conditionally Reprogrammed Patient-derived Cells

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Abstract

Many patients with muscle-invasive bladder cancer (BC) are either ineligible for or do not benefit from cisplatin-based chemotherapy, and there is an unmet need to estimate individuals' drug sensitivities. We investigated the suitability of conditionally reprogrammed (CR) cells for the characterization of BC properties and their feasibility for personalized drug sensitivity screening. The CR cultures were established from six BC tumors with varying histology and stage. Four cultures were successfully propagated for genomic, transcriptomic, and protein expression profiling and compared to the parental tumors. Two out of four CR cultures (urothelial carcinoma and small cell neuroendocrine carcinoma [SmCC]) corresponded well to their parental tumors and underwent drug sensitivity screening to identify novel drugs for the respective tumors. Both cultures were sensitive to standard BC chemotherapy agents (eg cisplatin and gemcitabine) and to conventional drugs such as taxanes and inhibitors of topoisomerase and proteasome. The SmCC cells were also sensitive to statins (eg, atorvastatin and pitavastatin). In summary, after confirming their representativeness and origin, we conclude that CR cells are a feasible platform for personalized drug sensitivity testing and might thus add to the approaches used to personalize BC treatment strategies.

Patient summary: We investigated the conditional reprogramming method for generating patient-derived bladder cancer cell cultures and studied their feasibility for planning personalized treatment strategies.

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Muscle-invasive bladder cancer (BC) is a mutationally heterogeneous malignancy with poor survival. Almost half of patients with this disease are ineligible for cisplatin-based adjuvant or neoadjuvant chemotherapy, and only a subset benefits from treatment [1]. Currently there are no validated means in the clinical setting to predict chemotherapy responses and/or clinical outcomes.

As BC is characterized by a lack of common druggable driver mutations and high intertumor heterogeneity, recapitulation of tumor characteristics ex vivo would offer tools for studying drug sensitivities for individual patients upfront and potential novel treatment approaches. Various patient-derived organoid and xenograft BC models have recently been established and used to identify personalized drug sensitivities [2]. Although these three-dimensional (3D) models replicate the tumor environment, it may not be feasible to use them for routine high-throughput diagnostics because of limitations associated with the culture complexity and the inability to accurately assess responses at the individual cell level.

Conditional reprogramming (CR) is a method that allows rapid expansion of malignant and normal epithelial cells without genetic manipulation. The CR approach has been used to generate patient-derived cultures from various neoplasms, including prostate cancer and respiratory papillomatosis [3,4]; however, the method has not been applied in human BC. The main challenge has been the overgrowth of benign epithelial cells, highlighting the need for stringent sampling of tumor material and validation of the origin of the cells via sequencing [5].

We applied the CR platform to generate patient-derived cell cultures from six BC patients undergoing cystectomy or transurethral resection (Fig. 1A). Four patients had pTaN0–T4N1 high-grade urothelial carcinoma (HG-UC), one patient had pT4aN1 small-cell neuroendocrine carcinoma (SmCC), and one patient had pT2bN1 primary bladder adenocarcinoma (Fig. 1A and B, Supplementary Table 1). The cultures were established according to a published CR procedure [6] with some modifications (Supplementary material). The cultures were considered stably established if they could be cultured for five passages, and after cryopreservation repropagated for follow-up analysis.

Four (3 HG-UCs and 1 SmCC) out of six CR cultures were successfully repropagated after cryopreservation (success rate 67%; Supplementary Table 1) and were denoted HG-Ta-CR, HG-T1-CR, HG-T4-CR, and SmCC-T4-CR. All the urothelial CR cultures shared typical CR culture morphology, while the SmCC-T4-CR cells had the expected smaller cellular size (Fig. 1C and E). HG-T1-CR and SmCC-T4-CR cells showed exponential growth during 30-d follow-up (Fig. 1D). Exome sequencing analysis showed that two of the cultures (HG-T1-CR and SmCC-T4-CR) retained the majority of mutations (eg, in *RB1*) found in the corresponding tumors, whereas the HG-Ta-CR and HG-T4-CR cultures did not, suggesting that normal

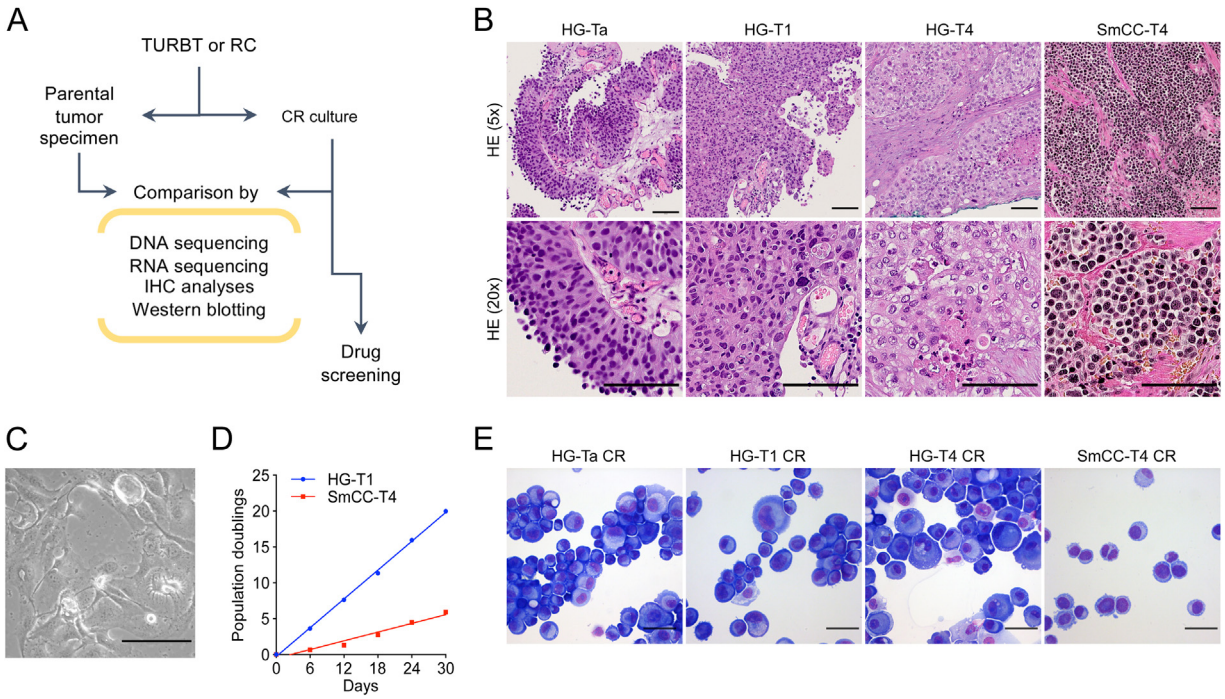

Fig. 1 – Establishment of conditionally reprogrammed (CR) cultures. (A) Overview of the study design. CR cultures were established from fresh tumor samples obtained from radical cystectomy (RC) or transurethral resection of bladder tumor (TUR-BT). The cultures and the corresponding tumor samples were characterized by immunohistochemical (IHC) analyses, Western blotting, and whole-exome DNA sequencing and RNA sequencing. Finally, high-throughput drug sensitivity screening was performed on the cancerous CR cultures. (B) Histology of parental tumors (low- and high-power magnifications). Three patients had high-grade (HG) urothelial carcinoma staged as pTa, pT1, and pT4aN1 (HG-Ta, HG-T1 and HG-T4, respectively; hematoxylin and eosin stain), and one patient had small cell neuroendocrine carcinoma (SmCC; Van Gieson stain) staged as pT4aN1 (SmCC-T4). Scale bars, 100 μm. Only tumors with stably established CR cultures are shown. (C) Representative phase-contrast microscopy image showing the typical cell morphology of a CR culture (example from HG-T1 CR). Scale bars, 50 μm. (D) Proliferation assays of HG-T1-CR and SmCC-T4-CR cultures. Both cultures showed exponential growth over the 30-d follow-up period. (E) May-Grünwald Giemsa-stained cytospin samples from CR cultures. SmCC-T4-CR cells showed an overall smaller cellular size compared to cells from urothelial carcinomas. Scale bars, 50 μm.

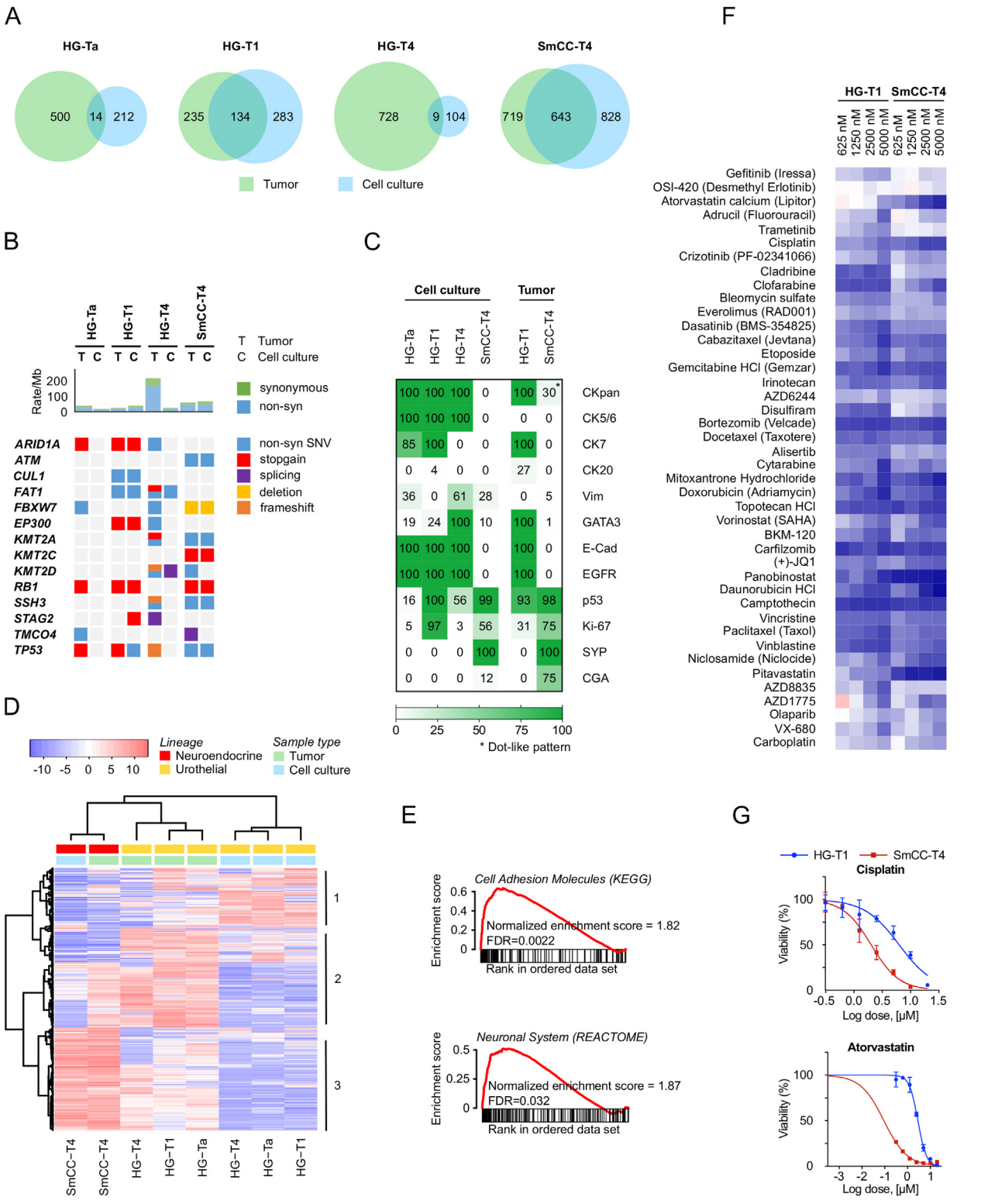

Fig. 2 – Characterization of conditionally reprogrammed (CR) cultures and their parental tumors. (A) For each tumor and cell-line pair, Venn diagrams depict the total number of mutations per sample with the number of co-occurring mutations shown in the overlapping circles. Tumor samples are depicted in green and cell cultures in blue. HG-T1-CR and SmCC-T4-CR cells retained the majority of mutations found in the corresponding tumors, whereas HG-Ta-CR and HG-T4-CR did not. (B) Top panel: Tumor mutation burden shown as synonymous (green) and nonsynonymous (blue) mutation rates per Mb. Bottom panel: The significantly mutated genes in the CR cultures (C) and in the corresponding tumors (T), as reported in The Cancer Genome Atlas BC cohort [11]. Only the exonic or splicing mutations found in either HG-T1 or SmCC-T4 tumors/cells are shown. Supplementary Fig. 1 and Supplementary Tables 2 and 3 show individual mutations also found in HG-Ta and HG-T4 tumors/cells. (C) Immunohistochemistry analysis of CR cultures and the HG-T1 and SmCC-T4 parental tumors shown as a heat map. The HG-T1 tumor shows characteristics of the luminal type, while the CR culture shows transition towards a more basaloid/squamous phenotype as observed by the expression of keratin 5/6 (CK5/6) and reduced expressions of keratin 20 (CK20) and GATA3 in the corresponding CR culture. The SmCC-T4 tumor and CR culture correspond to the neuronal type. The number indicates the percentage of positively stained cells. CGA = chromogranin A. (D) Gene expression clustering analysis shows distinct sample groups

epithelial cells had overgrown in these two cultures (Fig. 2A and B, Supplementary Fig. 1, Supplementary Tables 2 and 3).

On immunohistochemical analysis, HG-T1-CR cells showed strong keratin 5/6 expression, indicating a shift towards a more basaloid phenotype (Fig. 2C, Supplementary Fig. 2). In SmCC-T4-CR cells, strong expression of neuroendocrine markers and loss of keratins were detected, similar to the parental tumor (Fig. 2C, Supplementary Fig. 2). Both cancer-originating cultures were strongly p53-positive and showed a higher percentage of Ki-67-positive cells when compared to noncancerous cultures (Fig. 2C, Supplementary Figs. 2 and 3).

In RNA sequencing and gene set enrichment analysis, the expression of cell adhesion-related transcripts clearly differentiated the primary tumors from CR cells cultured in the absence of stromal cells (Fig. 2D and E). Genes representative of basal cell fate (eg, *KRT5*, *KRT6*, *KRT17*, and *KRT19*) were enriched in CR cultures of urothelial origin, while SmCC-T4-CR and the parental tumor were distinct from the other cultures and tumors on the basis of strong expression of neuroendocrine genes. The CR cultures showed very little gain of functionality compared to the primary tumors. However, there was some increase in translation-related pathways and ribogenesis. Furthermore, the cancer-originating cultures exhibited significantly higher expression of various cell cycle-related genes and lower expression of *CCND1/CCND2* (due to loss of *RB1*) compared to the noncancerous cultures (Supplementary Table 4).

Drug sensitivity screening performed on cancer-originating cultures showed sensitivity to platinum-based drugs, taxanes, topoisomerase inhibitors, and proteasome inhibitors, independent of the difference in the proliferation rates of the cell cultures (Fig. 2F, Supplementary Fig. 4). The HG-T1-CR cells were resistant to the EGFR inhibitor erlotinib (in line with the activating E322K mutation detected in *MAPK1*) while the SmCC-T4-CR cells were highly responsive to statins at low concentrations. The effects of cisplatin (standard treatment) and atorvastatin (because of a high statin response) were further validated with more detailed sensitivity measurements, with highly similar results compared to screening obtained (Fig. 2G).

In the current study we demonstrated the feasibility of establishing patient-derived BC CR cultures that retain the hallmark mutations of the primary tumor without significant phenotypic drift. Furthermore, the CR cultures were suitable for drug sensitivity screening. Exome sequencing analysis demonstrated that two of the four CR cultures characterized matched the corresponding tumor, as seen in the previous studies [3]. However, the other two CR cultures failed to retain the specific driver mutations found in their parental tumors, suggesting contamination by nonmalignant cells. Therefore, detailed selection of original tumor material and genomic analysis are crucial to confirm the origin of the established culture.

The results of personalized drug screening are of particular interest and might point to novel therapy targets for individual patients or subgroups. While clinically used platin-based compounds were effective in the CR cultures in vitro, we also observed high sensitivity of SmCC-T4-CR cells to statins, which could be a promising, well-tolerated, and low-cost candidate for further studies. The specific mechanism of statins depends on the type of statin used and the type of cancer cells. In case of small cell lung cancer, the mechanism seems to be HMG-CoA reductase inhibition of cholesterol biosynthesis and subsequent impairment of Ras signaling [7]. In addition, for some urothelial BC cell cultures, statins appear to induce cell cycle arrest and inhibit proliferation via the PPARγ signaling pathway [8].

While the results from the current study are encouraging, the data are limited by the small number of the cultures produced and potential intratumor heterogeneity. Culture conditions, including the culture media used and the absence of stromal and inflammatory cells, may also affect the tumor microenvironment and drug sensitivities [9,10]. Since conditioned media appear to be a prerequisite for successful culture, a more detailed characterization of the impact on drug sensitivities during the development of rapid 3D culture models needs to be tested in future studies.

Author contributions: Pekka Taimen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Critical revision of the manuscript for important intellectual content: Boström, Albanese, Poutanen, Taimen.
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relating to the tumor lineage (epithelial or neuroendocrine) and sample type (cell culture or primary tumor). Transcripts were selected on the basis of high variation across samples (n = 825): (1) genes representative of a basal cell fate, indicating enrichment of basal/tumor cells (eg, KRT5, KRT6, KRT17 and KRT19 positive) in the CR cultures compared to tumors; (2) cell adhesion molecules upregulated in tumors compared to the cell cultures (presumably due to lack of stroma in cell cultures); and (3) neuroendocrine genes upregulated in SmCC compared to UCs. (E) Gene set enrichment analysis further details the expression profiles that differentiate the sample classes. The top panel identifies cell adhesion as one of the main transcriptional themes differentiating primary tumors from the cell cultures (cluster 2 in D). The bottom panel clearly shows that both the SmCC tumor and SmCC-T4-CR retained strong neuroendocrine characteristics compared to the other samples (cluster 3 in D). (F) Heatmap of responses of the cancerous CR cultures to various drugs, including OSI-420 (desmethyl erlotinib), atorvastatin, the MEK1/2 inhibitor trametinib, a large group of commonly used chemotherapy agents (eg, cisplatin/carboplatin, taxanes), pitavastatin, and carboplatin. The intensity of the blue color correlates with sensitivity to a drug and reduced viability. Supplementary Fig. 4 shows the complete screening results. (G) Dose–response curves for HG-T1-CR and SmCC-T4-CR cells treated with cisplatin and atorvastatin. Both cultures were sensitive to cisplatin, while SmCC-T4-CR showed significantly reduced viability after treatment with atorvastatin compared to the HG-T1-CR culture. Data points denote the mean ± standard deviation (n = 3).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2019.06.016.

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