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expanded TICs in culture and thereafter injected them in larger numbers than those contained in the PDX transplants. Regarding intratumor heterogeneities, our PDXs and spheroids that derived from separate subregions of the same tumor were almost identical as others reported (5, 26, 27).

Second, the PDSX method allowed us to reduce variances in the tumor growth rate. As noted above, TIC spheroids consisted of only proliferating cancer epithelial cells. In case of PDXs, on the other hand, the engrafted tumor fragments upon passages were likely to be heterogeneous regarding the number of live TICs and their microenvironment (e.g., the extent of differentiation and coexisting stromal cells, respectively). In fact, we had significantly fewer mice excluded in the PDSX groups than in PDX upon preparations for drug-dosing tests, indicating that PDSX method is less wasteful in xenograft formation.

Third, and most importantly, PDSXs gave chemosensitivity data statistically more significant than PDXs in drug-dosing tests. Accordingly, it is expected that the clinical responses can be predicted more reliably with PDSX mice than with PDX. To date, many methods have been proposed that predict patient responses to cancer chemotherapeutics (28). These include mutational analyses, gene expression signature analyses, as well as *in vitro* sensitivity tests with cancer organoids similar to spheroids (14, 29). For example, colorectal cancer that retains intact Ras signaling with wild-type *RAS* genes responds to EGFR inhibitors at a high probability (25, 30). However, there is always a sizable fraction in the patient population that does not respond to the indicated regimen(s). Although it is beneficial to each patient if a regimen is efficacious, the patient will lose precious time and QOL if not. Thus, more reliable prediction methods are awaited. To this end, direct tumor grafts to immunodeficient mice (i.e., PDXs) have been proposed as a straightforward method of drug sensitivity evaluation personalized to the respective patients (3, 4).

Our current results provide a rationale for PDSXs as a more improved method than PDXs, overcoming the drawbacks of the latter. Namely, the results of PDSX drug-dosing tests demonstrated a strong correlation with the clinical responses (Figs. 3–5; Supplementary Figs. S6–S9), and paralleled with those of PDXs (Supplementary Table S3). Therefore, these results suggest a strong predictive power in chemosensitivity when applied to personalized prospective studies. To this end, our PDSX method also helps expedite preparation of test mice for drug-dosing tests. Namely, it took only 2 to 3 months to setup groups of PDSXs for testing several regimens. Notably, however, some types of cancer may be excluded that take very rapid downhill courses such as pancreatic cancer. It can be more practical to test the sensitivity of spheroids *in vitro* for such types. Along this line, *in vitro* cultures of spheroids/organoids have been proposed to be used in drug screening as well as in personalized chemosensitivity tests (9, 31). Such *in vitro* sensitivity tests may provide quicker, although limited, answers for a class of chemotherapeutics that directly target cancer cell proliferation. In other words, there are occasions in which xenografts can provide more practical prediction of the clinical courses as exemplified by irinotecan sensitivity in Fig. 4. Although irinotecan resistance was reported to correlate with the expression level of DNA topoisomerase I (32), our expression analysis did not provide enough data that allowed personalized predictions.

Taken together, the PDSX method should allow us to design personalized chemotherapy regimens for patients with advanced colorectal cancer in a prospective manner. We would like to propose introduction of personalized PDSX-based chemosensitivity tests as "personalized paraclinical prediction" (PPP) trials. For testing already established regimens, they can be designated as PPP phase 3.5X trials (X for xenograft) because the drugs had completed phase III clinical trials. When the tests can be performed using *in vitro* culture of spheroids/organoids, they may be PPP phase 3.5V (V for *in vitro*). If candidate drugs for repurposing are used, they can be PPP 1.5X or 1.5V trials, and so on. We encourage further discussion among those who participate in cancer chemotherapy development.

Notably, PDSXs have some limitations common to PDXs because both lack key immune responses and have different stromal microenvironment from that of human hosts. Technical improvements to humanize the mouse immune system and mimic the patient tumor microenvironment in mice appear to be in progress, although multiple difficulties still remain (4, 33, 34). Despite such limitations, PDX has been one of the best preclinical models owing to their predictive power for a variety of chemotherapeutics (3, 4).

In conclusion, we have demonstrated that PDSXs are reliable "paraclinical" models for personalized colorectal cancer chemotherapies. Our methods of TIC spheroids and PDSXs are straightforward, reliable, and well formulated. They also meet the timeline in most colorectal cancer clinical courses. In addition, PDSXs may be applied to chemotherapies of not only colorectal cancer, but also other types of cancer when the primary tumors are available. It is worth noting that applications of the PDSX method can be extended to evaluations of off-label uses of therapeutics and compassionate uses of yet-unapproved drugs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H. Maekawa, H. Miyoshi, T. Yamaura, M.M. Taketo
Development of methodology: H. Maekawa, H. Miyoshi
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Maekawa, H. Miyoshi, T. Yamaura, Y. Itatani
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Maekawa, H. Miyoshi, M.M. Taketo
Writing, review, and/or revision of the manuscript: H. Maekawa, H. Miyoshi, K. Kawada, Y. Sakai, M.M. Taketo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Itatani, K. Kawada, Y. Sakai
Study supervision: H. Maekawa, H. Miyoshi, M.M. Taketo

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References

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin 2017;67:177–93.
2. American Cancer Society. Colorectal cancer facts & figures 2014-2016. Atlanta, GA: American Cancer Society; 2014. p. 1–32.
3. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov 2014;4:998–1013.
4. Byrne AT, Alferez DG, Amant F, Annibali D, Arribas J, Biankn AV, et al. Interrogating open issues in cancer precision medicine with patient-derived xenografts. Nat Rev Cancer 2015;17:254–68.
5. Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, et al. Patient-derived xenografts undergo mouse-specific tumor evolution. Nat Genet 2017;49:1567–75.
6. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther 2011;10:1311–6.
7. Stebbing J, Paz K, Schwartz GK, Wexler LH, Maki R, Pollock RE, et al. Patient-derived xenografts for individualized care in advanced sarcoma. Cancer 2014;120:2006–15.
8. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc 2007;2:247–50.
9. Miyoshi H, Maekawa H, Kakizaki F, Yamaura T, Kawada K, Sakai Y, et. al. An improved method for cultureing patient-derived colorectal cancer spheroids. Oncotarget 2018;9:21950–64.
10. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161:933–45.
11. Fujii M, Shimokawa M, Date S, Takano A, Matano M, Nanki K, et al. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. Cell Stem Cell 2016;18:827–38.
12. Kondo J, Endo H, Okuyama H, Ishikawa O, Iishi H, Tsujii M, et al. Retaining cell-cell contact enables preparation and culture of spheroids composed of pure primary cancer cells from colorectal cancer. Proc Natl Acad Sci U S A 2011;108:6235–40.
13. Choi SY, Lin D, Gout PW, Collins CC, Xu Y, Wang Y. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Adv Drug Deliv Rev 2014;79–80:222–37.
14. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov 2017;7:462–77.
15. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goéré D, Mariani P, et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res 2012;18:5314–28.
16. Sausville EA. Preclinical models for anticancer drug development. In: Garrett-Mayer E, editor. Principles of anticancer drug development. New York, NY: Springer; 2011. p 89–114.
17. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61.
18. Luo FR, Yang Z, Dong H, Camuso A, McGlinchey K, Fager K, et al. Correlation of pharmacokinetics with the antitumor activity of cetuximab in nude mice bearing the GEO human colon carcinoma xenograft. Cancer Chemother Pharmacol 2005;56:455–64.
19. Oh BY, Lee WY, Jung S, Hong HK, Nam DH, Park YA, et al. Correlation between tumor engraftment in patient-derived xenograft models and clinical outcomes in colorectal cancer patients. Oncotarget 2015;6:16059–68.
20. Compton CC, Fielding LP, Burgart LJ, Conley B, Cooper HS, Hamilton SR, et al. Prognostic factors in colorectal cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000;124:979–94.
21. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol 2012;3:153–173.
22. Wu J. Statistical inference for tumor growth inhibition T/C ratio. J Biopharm Stat 2010;20:954–64.
23. Brown KM, Xue A, Mittal A, Samra JS, Smith R, Hugh TJ. Patient-derived xenograft models of colorectal cancer in pre-clinical research: a systematic review. Oncotarget 2016;7:66212–25.
24. Fichtner I, Slisow W, Gill J, Becker M, Elbe B, Hillebrand T, et al. Anticancer drug response and expression of molecular markers in early-passage xenotransplanted colon carcinomas. Eur J Cancer 2004;40:298–307.
25. Schütte M, Risch T, Abdavi-Azar N, Boehnke K, Schumacher D, Keil M, et al. Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. Nat Commun 2017;8:14262.
26. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med 2015;21:1318–25.
27. Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, Batra RN, et al. A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. Cell. The Authors; 2016;167:260–74.
28. Friedman AA, Letai A, Fisher DE, Flaherty KT. Precision medicine for cancer with next-generation functional diagnostics. Nat Rev Cancer 2015;15:747–56.
29. Calon A, Lonardo E, Berenguer-Llergo A, Espinet E, Hernando-Momblona X, Iglesias M, et al. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. Nat Genet 2015;47:320–9.
30. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 2015;26:13–21.
31. Weeber F, Ooft SN, Dijkstra KK, Voest EE. Tumor organoids as a pre-clinical cancer model for drug discovery. Cell Chem Biol 2017;24:1092–100.
32. Hammond WA, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol 2016;8:57–84.
33. Morton JJ, Bird G, Refaeli Y, Jimeno A. Humanized mouse xenograft models: Narrowing the tumor-microenvironment gap. Cancer Res 2016;76:6153–8.
34. Francone TD, Landmann RG, Chen C-T, Sun MY, Kuntz EJ, Zeng Z, et al. Novel xenograft model expressing human hepatocyte growth factor shows ligand-dependent growth of c-Met-expressing tumors. Mol Cancer Ther 2007;6:1460–6.

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Molecular Cancer Therapeutics

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