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Basic Study

Individual chemotherapy for patients colorectal cancer based on patient-derived tumor-like cell clusters

This study explores the application of the PTC model in personalized chemotherapy for colorectal cancer. The overall experimental design is systematic, and the data are robust. Particularly noteworthy is the rich microscopic images presented, which visually demonstrate the diversity of PTCs and their response patterns to drugs—a very convincing approach. The authors successfully revealed the heterogeneous responses of different patients to chemotherapy regimens in vitro and proposed that PTC testing may help optimize clinical protocols, a line of thought with clear research value. However, upon closer reading, I also found several issues that warrant further clarification.

I. The standardization details of TC culture and drug sensitivity testing: The paper mentions using an "optimized efficiency concentration," but doesn't explain how this concentration was determined. I'm personally curious whether this concentration was set with reference to clinical blood drug concentrations or based on preliminary gradient experiments. After all, different drugs have significantly different in vivo metabolic characteristics; if only a single fixed concentration is used, can it truly simulate the patient's actual drug exposure level during treatment? I hope the authors can supplement the explanation of the basis for the concentration selection and appropriately discuss the potential limitations of this strategy.

II. Regarding the evaluation method for drug efficacy—defining cell killing efficiency (KE) based on changes in the area of the PTC (percutaneous

transcellular matrix)—while technically feasible, its underlying biological significance may require further confirmation. For example, to what extent does area reduction actually represent cell death? Is it possible that cell clusters are simply deaggregating without actual cell death? It is recommended that the authors perform a correlation analysis on the image analysis results with classic cell viability assays such as CellTiter-Glo used in the article in typical cases to verify the reliability of the imaging-quantitative method.

III. The discussion section's mention of FOLFOXIRI's toxic side effects is necessary, but I believe it could be explored further. Since a dual-drug regimen is suggested for some patients to reduce toxicity, could existing in vitro efficacy data be combined with known toxicity profiles to further explore how to integrate and analyze these data to make a more reasonable clinical trade-off between efficacy and safety? Such extended discussion would significantly enhance the clinical reference value of the article's conclusions.

Reply:

I. The drug concentration of fluorouracil, oxaliplatin and irinotecan was 2, 4, 1 μ M, that is, the optimized Efficacy Concentration in the original literature as “Yin S, Xi R, Wu A, et al. Patient-derived tumor-like cell clusters for drug testing in cancer therapy[J]. *Science Translational Medicine*, 2020, 12(549): eaaz1723.”. Also, we discussed the limitation.

II. Our description of the method was inaccurate, changes in transcellular matrix area cannot be directly used to define cell killing efficiency (KE) or determine cell death. the plates were screened again with the Nikon Ti-U microscope system to evaluated drug effect by measuring the change of microsphere area. It indicated that the PTCs were sensitive to drugs when the ratio PA, representing area of PTC after drug treatment to that before. The cell viability after applying a drug A was calculated by the following

formula: $P_{Ai} = \frac{S_{Ai,t1}}{S_{Ai,t0}}$, $P_A = \frac{1}{n} \sum_{i=1}^n P_{Ai}$. The formula for calculating the PAi, the efficacy evaluation index of the microtumor drug sensitivity detection system; S refers to the area of the PTC in a well, n refers to the number of replicate wells, and t0 and t1 refer to time points. B. Depiction of the classification of PTC drug sensitivity microtumors; the cut-off of the PA was 0.7.

III. We have provided more discussion.