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Determination of the cutoff value for drug efficacy in MTSs model

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MTS

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Abstract

Determination of clinical drug concentrations in the MTS model to predict patient-relevant chemotherapeutic response.

Guidelines

The drug testing of patient-derived MTSs was conducted in 96-well cell culture plates following the previously described procedure. Briefly, the collected MTSs were centrifuged at 800 rpm at 4 °C for 3 minutes, washed with PBS, and resuspended in SG-Medium. Approximately 50 MTSs in a volume of 50 µL of SG-Medium were seeded into each well of 96-well cell culture plates. Next, 50 µL of SG-Medium containing defined concentrations of the drugs was added to each well. The plates were incubated at 37 °C in an incubator with 5 % CO2. After 48 hours of culture, 100 µL Celltiter-Glo reagents was added to the culture medium and cell viability was measured using Synergy plate reader (BioTek). The SR70% (or IC30) and growth-inhibitory curves of MTSs were analyzed using GraphPad Prism 10.0 software.

Materials

- 96-well cell culture plates
- SG-Medium
- PBS
- Celltiter-Glo reagents
- Synergy plate reader (BioTek)
- GraphPad Prism 10.0 software

Determination of the cutoff value for drug efficacy in MTSs model

1 We determined the cutoff point for MTSs-drug sensitivity based on the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. For analysis purposes, we classified the RECIST criteria into two groups using a cut-off point of 70%, defining complete response (CR) or partial response (PR) as effective and stable disease (SD) or progressive disease (PD) as ineffective. Accordingly, after treating MTSs with drugs at their effective concentration (EC) at IC30, MTS survival rate (SR) was classified into two categories: ineffective if SR ≥ 70% and effective if SR 3c 70%. The formula used to calculate MTS survival rate after drug “a” treatment is as follows: SR_a = cell viability value of the drug “a” treatment / cell viability value of the negative control (NC) with vehicle treatment. In our MTSs model, when a patient received neoadjuvant chemotherapies and Magnetic resonance imaging (MRI) indicated SD or PD while having cell SR ≥ 70%, it indicates that the drug testing using MTSs can correctly predict the clinical outcomes.

2 The drug testing of patient-derived MTSs was conducted in 96-well cell culture plates following the previously described procedure. Briefly, the collected MTSs were centrifuged at 800 rpm at 4 °C for 3 minutes, washed with PBS, and resuspended in SG-Medium. Subsequently, approximately 50 MTSs in a volume of 50 µL of SG-Medium were seeded into each well of 96-well cell culture plates. Next, 50 µL of SG-Medium containing defined concentrations of the drugs was added to each well. The plates were incubated at 37 °C in an incubator with 5 % CO2. After 48 hours of culture, 100 µL Celltiter-Glo reagents was added to the culture medium and cell viability was measured using Synergy plate reader (BioTek). The SR70% (or IC30) and growth-inhibitory curves of MTSs were analyzed using GraphPad Prism 10.0 software. After integrating growth-inhibitory curves from 30 treatment-naïve BRC patients, the determination of S R70% cutoff value was based on the efficacy rate (EFR) that closely approximated the overall response rate (ORR). The efficacy rate (EFR) represents the proportion of recruited patients (N=30) that was closest to the ORR of this drug previously reported in relevant clinical trials with fixing the SR70% cut-off value. The SR70% cutoff values of drugs were listed as below.
Regimens Overall response rate (ORR) and Efficacy rate (EFR) with SR70% cutoff values are provided in the table below.

3 In vivo, Capecitabine, an oral prodrug of 5-fluorouracil (5-Fu), undergoes enzymatic activation mediated by three enzymes to convert into its active form, 5-Fu. Due to its short half-life and requirement for metabolism in vivo, capecitabine is replaced with 5-Fu to be employed for cytotoxicity assays in MTS model in this study. Additionally, palifosfamide, a metabolite derived from cyclophosphamide, has also been employed for cytotoxicity assays in the MTS model.