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MTSs generation, characterization, collection and passage V.1

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MTS



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We use this protocol and it's working

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Abstract

A comprehensive overview of clinical sample sourcing, processing protocols, and standardized procedures for collection, cryopreservation, and in vitro expansion (including passaging) in the context of MTS generation.

Sphere growth medium (SG-Medium)

- 1 The 200 mL SG-Medium used induce MTSs generation in this study was prepared by combining 132 mL of Advanced DMEM/F12 (Thermo Fisher Scientific) with the following components: 40 mL of Wnt3a-conditioned medium, 20 mL of R-spondin-1-conditioned medium⁶⁰, 4 mL of a 50× B27 supplement (Thermo Fisher Scientific), 2 mL of nicotinamide (1 M in DPBS, Sigma-Aldrich), 2.0 µL Noggin (100 ng/ml, PeproTech), 500 µL of N-acetyl-L-cysteine (500 mM in water, Sigma-Aldrich), 400 µL of hydrocortisone (250 µg/ml, Sigma-Aldrich), 200 µL of β-estradiol (100 µM, Sigma-Aldrich), 200 µL of Forskolin (10 mM in DMSO, Sigma-Aldrich), 400 µL of Primocin (50 mg/ml, Invivogen), 10 µL of Y-27632 (100 mM in DMSO, AbMole Bioscience), 100 µL of Heregulin β1 (10 µM in DPBS-B, PeproTech), 100 µL of human FGF-10 (40 µg/ml in DPBS-B, PeproTech), 20 µL of A83-01 (5 mM in DMSO, Tocris Bioscience) and 2.0 µL of human EGF (500 µg/ml in 0.1% DPBS-B, PeproTech). This complete medium can be stored for up to one week when kept at 4 °C.

Generation and collection of MTSs

- 2 For generation of MTSs derived from clinical BRC tissues, the surgical or biopsy specimens were immediately immersed in ice-cold phosphate-buffered saline (PBS) supplemented with penicillin-streptomycin (100 U/mL) and transported to the laboratory in 24 hours. Necrotic regions and adipose tissue were excised as possible, followed by washing with PBS. The excised tissues were then minced into small pieces and subjected to enzymatic digestion using collagenase I, II, IV along with DNase (Thermo Fisher Scientific) at 37 °C for 1-1.5 hours. In case of any visible red color appeared during sample preparation, erythrolysis was performed with RBC lysis buffer (Invivogen) before the washing step. Typically, 0.5 mL of lysate could digest one piece of biopsy samples with a total weight of approximately 50 mg. The digestion mixture was pipetted every 15 minutes to facilitate cell release. The dissociated cells were collected by passing through a 40 µm filter and then centrifuged at 1,200 rpm for 5 minutes at 4 °C. After centrifugation, the cells were resuspended in selective medium (Sel-Medium), the DMEM/F12 selective medium containing 15 mM HEPES buffer (STEMCELL Technologies). The resuspended cells were then seeded onto one MWC-chip. Following 2 days of culture in Sel-Medium, the medium was replaced with SG-Medium for further culturing of cells for an additional 2-3 days in an incubator at 37 °C, with 5% CO₂. Subsequently, MTSs larger than 30 µm diameter formed within microwells amidst numerous single cells or cell debris. The capacity of MTSs formation on the cell-chip was quantified using the following equation: MTSs formation rate on microwell cell-chip (MFR) = (Number of MTSs on a MWC-chip / Total number of wells within a MWC-chip) × 100%. The success rate of MTS

generation from clinical samples used in this study was determined as follows: (Number of samples with MFR > 5% / Total number of samples used in this study) × 100%. To capture the growth dynamics of MTSs, cells cultured in SG-Medium were imaged every 5 minutes using LumaScop 620 (Etaluma) over a total duration of 96 hours. The resulting videos were created and analyzed using LumaScop 620 (Etaluma).

The generation of MTSs in this study utilized a panel of seven BRC cell lines, namely MDA-MB-231 (M231), MCF7, MDA-MB-435S, BT-549, AU-565, MDA-MB-453 and MDA-MB-435. Following trypsinization of the cells from monolayer culture, they were collected and seeded in the MWC-chip for subsequent culture in the Sel-Medium for 3 days, followed by an additional two-day culture under SG-Medium conditions to induce MTSs formation.

After 5 days of culture, the SG-Medium was aspirated and all cells, including MTSs and single live cells, as well as cell debris, were collected as a mixture from the microwells by washing with PBS. The MTSs ranging in diameter from 30 to 70 µm were sorted using hydrodynamics actuated cell sorting (HACS) technology, or individual MTS and other residual cells (RCs) within a microwell were accurately picked separately using CytoSTAR (Livingchip) for subsequent experiments.

Passage of MTSs

- 3 The collected MTSs were trypsinized into single cells using TrypLe (Thermo Fisher Scientific). Following a wash with F-12 medium (Thermo Fisher Scientific), the cells were centrifuged at 1,200 rpm for 5 minutes. Single cells derived from MTS were resuspended in SG-medium and seeded onto new MWC-chips (diameter: 80 µm) at a density of approximately 5–10 cells per well. Following approximately 5 days of culture, the MTS proliferated to reach a diameter of about 80 µm. At this stage, the MTS were harvested for subsequent passage.

The characterization of MTS

- 4 To demonstrate the formation of MTSs, all isolated cells from BRC tissues were divided into two equal pools. One pool of cells was stained with green fluorescent dye (DIO, YEASEN) and the other pool of cells were stained with red fluorescent dye (DID, YEASEN). The two populations of labeled cells were then mixed in a 1:1 ratio. Subsequently, the mixed cell suspension was seeded onto MWC-chips and cultured for a period of 5–6 days to induce MTSs formation. The resulting MTSs were stained with either a single color (green or red) or double colors (green and red merging to form yellow), followed by identification using an inverted fluorescence microscope (Eclipse Ti-Eclipse, Nikon). The quantification of cell numbers within each MTS and each microwell was performed by enumerating antifade mounting medium with Hoechst 33342 stained nuclei (Beyotime). The diameter of MTSs was measured by Image J software (National Institutes of Health). Cell proliferation ability was measured by Click-it EdU imaging kits (Thermo, Alexa Flour 594). In brief, cells on MWC-chip were



incubated in SG-Medium with 20 mM EdU for 48 hours. After 4% paraformaldehyde fixation and 0.5% Triton X-100 permeabilization, cells were incubated in Click-iT Plus reaction cocktail for 30 minutes. Nuclei were marked by 5 mg/mL Hoechst 33342 overnight. Images were captured with Nikon Ti-U microscope system.

Protocol references

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