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Cell proliferation related assays

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Abstract

Clonogenic survival assay in soft agar and Matrigel and cell proliferation assay of MTS-M231 cells

Materials

- Parental M231 cells
- M231-derived MTSs cells (M231-MTSs)
- Patient-derived MTSs and RCs
- Trypan blue staining (Beyotime)
- 35-mm dishes
- 0.5% agarose
- Complete DMEM medium
- 4% low-melting agarose
- Matrigel
- SG-Medium
- Zoom-stereo microscope SZX16 (OLYMPUS)
- PBS
- DMEM/F12 basal medium
- Glutamine
- EGF
- bFGF
- B27
- 24-well plates
- 96-well plate
- CellTiter-Glo (CTG) reagent (Promega)
- Synergy plate reader (BioTek)

Clonogenic survival assay in soft agar and Matrigel

1 The tumorigenicity of the tumor cells was measured using a colony-forming assay. Parental M231 cells and M231-derived MTSs cells (M231-MTSs), as well as patient-derived MTSs and RCs derived from the same patient tumor, were trypsinized, harvested, and counted following trypan blue staining (Beyotime). For clonogenic assay in soft agar, 35-mm dishes were coated with 0.5% agarose supplemented with complete DMEM medium. Once the bottom layer was solidified, 2,000 viable single cells in 1.6 mL of complete medium were mixed with 0.15 mL 4% low-melting agarose and evenly spread in the dishes for solidification purposes. For clonogenic assay in Matrigel, 2,000 patient-derived MTSs and RCs cells were resuspended in 50 µL SG-Medium and combined with an equal volume of Matrigel on ice. After dispensing 100 µL of the cell suspension into each well of a 24-well plate, solidification was achieved by incubating at 37°C for 30 minutes and immersed in SG-Medium. Subsequently, the dishes were incubated at 37 °C in an atmosphere containing 5% CO_2 for 14 days. Finally, colonies were enumerated and visualized under a Zoom-stereo microscope SZX16 (OLYMPUS).

Sphere Formation assay in suspension culture

2 The sphere formation assay is a well-established *in vitro* test employed for the identification of cancer stemness. The parental and MTS-M231 cells were trypsinized, washed with PBS, and resuspended in serum-free medium consisting of DMEM/F12 basal medium, 1× Glutamine, 20 ng/ml EGF, 10 ng/ml bFGF, and 2% B27. Subsequently, 2,000 cells were seeded and cultured in low attachment 24-well plates. After 10 days of culture, sphere was formed, photographed and analyzed.

Cell proliferation assay of MTS-M231 cells

3 A total of 800 MTS-M231 and parental M231 cells were seeded in one well of a 96-well plate for monolayer culture in 200 µL SG-Medium. At time points of 0, 1, 2, 3, 4, 5, and 6 days, the medium was aspirated and replaced with reaction medium containing 100 µL SG-Medium and 100 µL CellTiter-Glo (CTG) reagent (Promega) in one well for cell viability assay. The cells were then incubated at room temperature for one hour, followed by obtaining viability readings using the Synergy plate reader (BioTek).