Cytotoxic effects of CA-Mel and proton radiation on HCT-116 tumoral spheroids

Cell line: HCT116 is a human colon cancer cell line used in therapeutic research and drug screenings. HCT116 cells are used in a variety of biochemical investigations involving colon cancer proliferation and corresponding inhibitors. The cell line has been used in tumorigenesis studies. Cells were grown in DMEM (Dulbecco’s Minimum Essential Medium) supplemented with 10% fetal calf serum (FCS), 100 units/mL of penicillin, and 100 µg/mL of streptomycin at 37 °C in a humidified incubator under an atmosphere containing 5% CO₂.

MTT analysis: The cell viability was assessed using a MTT assay. First, the HUJ cells were plated into 24 well plates. After 24h of growing cells in the presence of the nanoplatform, the medium and the discs were removed from the wells, which were then incubated with a final concentration of 1mg/mL of MTT. After 4 h, the medium was removed and DMSO was added to dissolve the formed crystals. The optical absorbance was recorded at 570 nm using the plate reader Mithras LB 940 (Berthold, Germany) and the absorbance values of blank wells (only DMSO) were subtracted in order to calculate the cell viability.

Spheroid Formation and Analysis: A concentration of 5000 cells/well of HCT-116 cells was seeded. A final volume of 200 µL of cell suspension was placed in each well of a clear, round bottom, ultra-low attachment 96-well microplate (Corning, NY, USA). After this, the plate was centrifuged for 2 min and then incubated at 37 °C for up to 5 days. Spheroid formation was confirmed by observing the plate under a light microscope (Olympus CX23 Binocular Microscope, Düsseldorf, Germany). Spheroids were monitored daily and the incubation medium was replaced every 3 days.

ATP Assay: ATP levels in the treated spheroids were assessed, as will be described below. Here, 100 µL of medium was removed from each well, then the remaining 100 µL with the spheroid was transferred into an opaque 96-well plate. After this, 100 µL of CellTiter-Glo® reagent (Promega, Madison, WI, USA) was added onto the spheroids, which were incubated at room temperature for 10–15 min under thorough shaking to make sure that the spheroids were broken. Finally, the luminescence of the cells was measured using the plate reader.

Conclusions

• The formed spheroids of tumoral nature were affected significantly by our scheme of treatment showing the benefits that occur with the use of combination treatments which can reduce toxicity and possible appearance of internal lesions.

• The antimicrobial activity of the hybrid CA-Mel peptide applied on the spheroids was proved to be significantly enhanced by the presence of proton irradiation. Moreover, the sinergy between the two types of treatment should be studied more in case other combinations could prove even more appropriate.

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