Supporting information

Tumor-targeted magnetic micelles for magnetic resonance imaging, drug delivery, and overcoming multidrug resistance

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1. Experimental details

1.1 Determination of IC_{50} values for C666-1 cells

C666-1 cells were used as the parent cells in this experiment, the IC_{50} value of C666-1 cells needs to be determined first. The IC_{50} value of C666-1 was determined by MTT colorimetric assay. The specific experimental procedures are as follows:

1. C666-1 cells in 200 μL of logarithmic growth were seeded on 96-well plates at a cell density of 1×10^5 cells per well.
2. After 24 hours of attachment, the old solution was discarded and new DMEM medium (containing 10% FBS) containing different concentrations of docetaxel (10, 20, 30, 40, 50, 60, 70 and 80 μg/mL) was added, and three complex wells were designed.
3. After 48 hours, discard the old solution, add 200 μL new DMEM medium (containing 10% FBS) and 50 μL 5 mg/mL MTT solution, and incubate for 4 hours. After removal of the supernatant, staining was terminated by adding 150 μL DMSO to each well. The absorbance OD value of each well was measured by microplate reader at a wavelength of 490 nm, and the cell survival rate was calculated according to the OD value and the cell survival rate histogram was drawn. The IC_{50} value was calculated by Graphpad Prism 8 software to be 17.99 μg/mL. Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = \left({}^{OD490sample}/_{OD490control}\right) \times 100\%$$

1.1.2 Screening of drug-resistant cell lines

The principle of constructing drug-resistant strains is that drugs induce mutations in some genes in cells, resulting in drug resistance, which is a process of natural selection. Exposure of nasopharyngeal carcinoma cells by long-term exposure to low-dose drugs [1, 2]:

1. For the screening of C666-1/DOC cells, C666-1 cells in the logarithmic growth phase were cultured in six-well plates at a cell density of 1×10^6 cells per well.
2. Docetaxel was added when the cell density reached 70-80%. In the first stage, drug was added at 1/8, 1/4 and 1/2 of the IC_{50} of parental cells (17.99 μg/mL) in order to avoid a large number of cell death at the beginning of drug addition.
3. After 48 hours, the cells were passaged, and if a large number of cells died, the cells were washed twice with PBS buffer before trypsin digestion. The cells were then transferred to clean wells in a new six-well plate for further culture while the cell status was observed.
4. If the density did not grow to 70-80% by the next day, the culture medium was changed and the culture was continued. When cell growth reached 70 to 80%, the same dosing treatment was continued. The procedure was repeated to screen for stable resistant strains.
5. If C666-1 cells can grow normally at a specific concentration and there is basically no cell death, increase the concentration of docetaxel for dosing. Until the cells can grow stably and can be passaged at a specific drug concentration.
6. C666-1 cells were incubated with different concentrations (10, 20, 30, 40, 50, 60, 70 and 80 μg/mL) of docetaxel. The absorbance of each well in a 96-well plate was measured by a microplate reader at 490 nm wavelength. The IC_{50} value was calculated by Graphpad Prism 8 software. At the same time, the survival rate of parental cells treated with docetaxel in this concentration range was compared to check the drug resistance effect. After drug culture, the survival rate of C666-1/DOC cells was statistically different from that of C666-1 cells ($P < 0.05$).
7. In the whole process of adding drugs, the state of cells is the key to culture, and only under the condition of good state can the drug be added for the purpose of drug screening. Finally, C666-1/DOC cells with IC_{50} value of 43.58 μg/mL were selected as the drug resistant strain in this study. In order to maintain the drug resistance of C666-1/DOC cells, taking the IC_{50} concentration of C666-1/DOC as a reference and without significant effect on cell growth, 10 μg/L docetaxel was added to the DMEM medium during cell culture.

2. Supporting Figure

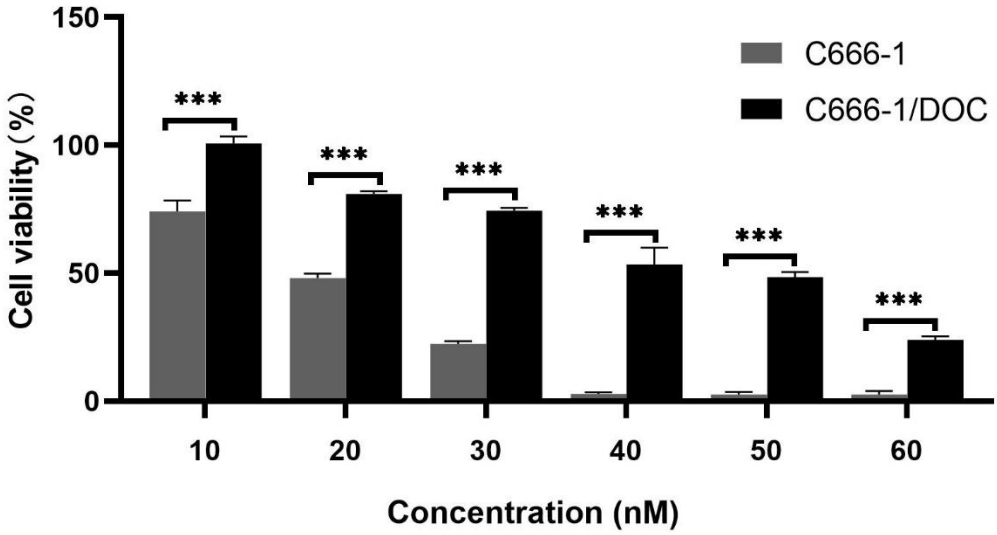

Fig. S1. Cell viability of C666-1 cells and C666-1/DOC cells.

3. References

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(2) Zhou Z, Zhang L, Xie B, Wang X, Yang X, Ding N, Zhang J, Liu Q, Tan G, Feng D, et al. FOXC2 promotes chemoresistance in nasopharyngeal carcinomas via induction of epithelial mesenchymal transition. Cancer Lett, 2015, 363(2): 137–145.