Proliferation constant study of the CRL-1888 mouse tumor cell-line for the application of hyperthermia animal model

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Purpose

This study is to investigate the proliferation characteristics of the CRL-1888 mouse tumor cell line at eleven different temperatures, which is very useful parameter for designing an interstitial thermo-seed hyperthermia animal tumor model.

Methods

The CRL-1888 mouse tumor cell line was acquired from the American Type Culture Collection (USA). Cell medium was prepared with the mixture of the Dulbecco's Modified Eagle's Medium-low glucose (Gibco, USA), 10% Fetal Bovine Serum (Gibco, USA), 1% Penicillin-Streptomycin (Gibco, USA) and 1% of the 200mM L-glutamine (Gibco, Japan). The CRL-1888 Cells were taken out from the liquid nitrogen storage and thawed in an isothermal chamber (Koma Biotech, Korea). Cells were separated from the medium using a centrifuge (MF80, Hanil, Korea). The cells were cultured in a CO_2 incubator (BINDER CB-50, Germany) up to the number of $6x10^6$. Ten thousand CRL-1888 cells were selected and put them into a φ100mm cell-culturing Petri dish and then incubated at eleven different temperatures; i.e. 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46□ and for seven different incubation times; i.e. 24, 48, 72, 96, 120, 144 and 168hours. Every culturing test was repeated for three times. Microscopy assay was tested with optical microscope (CK40-F200, Olympus, Japan) at the x200 magnification. Cellular viability was tested using the Trypan blue exclusion method. The number of cells in 10μl were measured with a Hemocytometer(Marienfeld, Germany).

Results

The number of cells incubated at 37□ for 24hous were $2.9x10^4$. This figure indicated that the cells were proliferated about 2.9 times more than the initial number of $1x10^4$ at this condition. At the same temperature, the cells were proliferated about 66 times more than the initial one during 168 hours incubation. Figure 1 shows the increasing curves of the CRL-1888 cells at eleven different temperatures. The diagrams show that the proliferation curves of the CRL-1888 mouse tumor cell line are well in accord with the theoretical formula[1] $LogN = LogN_0 + kt$. Where N is a measured number of cells, N_0 is the initially inoculated number of cells and k is the proliferation characteristic constant which was calculated from the interpolated slope of the proliferation curves. The maximum value of the proliferation constant k was 0.021 at 39℃ incubation test. However, it was 0.011 for normal incubation test temperature of 37℃. These results were well in accord with the results of the previous microscopic observation study.[1] And the value of the proliferation constant k was conversed to negative value at the temperature above 42℃. Therefore, the effectiveness of hyperthermia treatment for CRL-1888 could be expected at the temperature above 42℃.

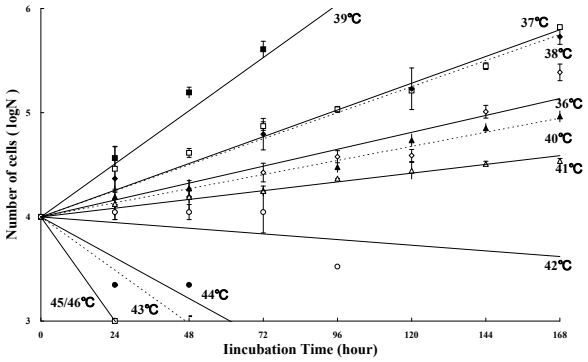

Figure 1. Cell counts of the CRL-1888 after incubations at eleven different temperatures.

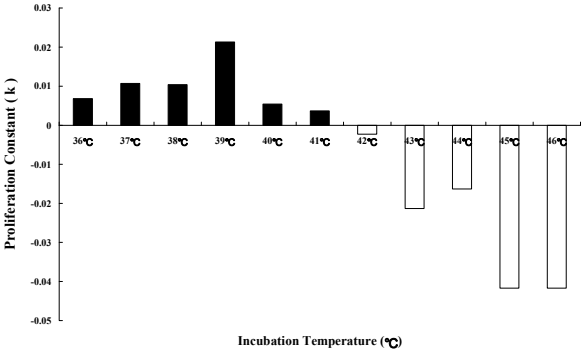

Figure 2. Graphs of the proliferation constant k of the CRL-1888 mouse tumor cell line at eleven different testing temperatures. The k value is conversed to negative above 42℃.

Conclusion:

In this proliferation study, we had found that the conversion temperature of the proliferation constant k of the CRL 1888 cell line is 42℃. This conversion temperature would be a very useful parameter for designing a thermo-seed size and heating characteristics for hyperthermia of the animal tumor model.

Acknowledgement

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References

1. Eun Mi Hwang and Young Kon Kim, "Proliferation characteristics of the CRL-1888 mouse tumor cell line for hyperthermia analysis with respect to culturing temperatures", Biomaterials Research Vol. 12 No. 1, 29-34, 2008