Title

Histological and proteomic analysis for the evaluation of perfusion culture system of spheroids (13words)

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Introduction

Constant nutrient supply by blood vessels is important for tumor maintenance and growth. In the static culture, the medium is changed every several days, which is different from the natural environment. Perfusion culture provides a constant supply of fresh medium to the tumor tissue and eliminates waste products. The comprehensive analysis allows us to evaluate the usefulness of perfusion culture from multiple perspectives at the molecular level. However, there are few cases in which the difference between static and perfusion culture was compared in a comprehensive analysis.

Methods

In this study, we investigated the characteristics of perfusion culture. The spheroids were used instead of tumor tissue. The patient-derived undifferentiated polymorphous sarcoma cell line NCC-UPS4-C1 was used for the fabrication of the spheroids. Histological observations and proteomic analysis were performed to examine the properties of perfusion-cultured spheroids. For histological observation, spheroids were paraffin-embedded, sectioned, and stained with hematoxylin-eosin. Phase transfer surfactant was added to the spheroids, and proteins were extracted using an ultrasonic disrupter. The extracted proteins were enzymatically digested and examined by LC-MS/MS.

Results

Histological observations showed that perfusion culture prevented necrosis inside the spheroids. LC-MS/MS confirmed the expression of 2400 proteins. Among the 1000 proteins for which comparative analysis was possible with high accuracy, we identified proteins whose

expression was upregulated by perfusion culture. Prevention of internal necrosis by perfusion culture is important to distinguish between cell death by the culture stress and the anti-cancer drugs. One of the proteins up-regulated by perfusion culture is the E3 ubiquitin-protein ligase HUWE1, which ubiquitinates the anti-apoptotic factor Mcl-1, and its expression may influence drug sensitivity testing.

Conclusions

The perfusion culture is a promising method for *in vitro* drug sensitivity tests using spheroids. To further demonstrate the practical utility of perfusion culture, we plan to analyze the perfusion-cultured tumor tissues.

(299words)