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Optimization of the tissue source, malignancy, and initial substrate of tumor cell-derived matrices to increase cancer cell chemoresistance against 5-fluorouracil

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ABSTRACT

The low chemoresistance of *in vitro* cancer cells inhibits the development of new anti-cancer drugs. Thus, development of a new *in vitro* culture system is required to increase the chemoresistance of *in vitro* cancer cells. Tumor cell-derived matrices have been reported to increase the chemoresistance of *in vitro* cancer cells. However, it remains unclear how tissue sources and the malignancy of cells used for the preparation of matrices affect the chemoresistance of tumor cell-derived matrices. Moreover, it remains unclear how the initial substrates used for the preparation of matrices affect the chemoresistance. In this study, we compared the effects of tissue sources and the malignancy of tumor cells, as well as the effect of the initial substrates on chemoresistance against 5-fluorouracil (5-FU). The chemoresistance of breast and colon cancer cells against 5-FU increased on matrices prepared with cells derived from the corresponding original tissues with higher malignancy. Moreover, the chemoresistance against 5-FU was altered on matrices prepared using different initial substrates that exhibited different characteristics of protein adsorption. Taken together, these results indicated that the appropriate selection of tissue sources, malignancy of tumor cells, and initial substrates used for matrix preparation is important for the preparation of tumor cell-derived matrices for chemoresistance assays.

1. Introduction

The development of effective anti-cancer drugs is crucial for cancer therapy; as a result, trials to develop new anti-cancer drugs have been extensively performed [1]. Despite extensive efforts, the development of a new anti-cancer drug remains difficult due to the lack of an effective development process [2]. The developmental process of an anti-cancer drug generally involves *in vitro* screening, *in vivo* testing using animal models, and clinical trials. Although a cell culture system is widely used for *in vitro* screening, *in vitro* cancer cells exhibit considerably lower chemoresistance compared to *in vivo* cancer cells, which results in faulty results and causes inadequate screening. Thus, a new cell culture system that increases the chemoresistance of cancer cells has been proposed to enable reliable *in vitro* anti-cancer drug screening [2–4].

Focusing on the extracellular matrix (ECM) is one of the important approaches used to increase the chemoresistance of *in vitro* cancer cells because the ECM regulates many cell functions, including cell survival, proliferation, and chemoresistance [5]. In particular, several reports have indicated that cell-derived matrices, which consist of ECM proteins deposited by cultured cells and are prepared as new cell culture substrates by the removal of cellular components from the culture (decellularization), can increase the chemoresistance of cancer cells *in vitro* [6–8]. We previously prepared “staged tumorigenesis-mimicking matrices” that mimic *in vivo* ECM surrounding tumor cells at various stages of malignancy [9]. We also compared the chemoresistance of a breast cancer cell line, MDA-MB-231, against 5-fluorouracil (5-FU) on staged tumorigenesis-mimicking matrices. The chemoresistance of MDA-MB-231 cells against 5-FU changed on matrices derived from cells with different degrees of malignancy [9] indicated that the malignancy of tumor cells for matrix preparation can affect the chemoresistance on tumor cell-derived matrices. However, it still remains unclear how matrices derived from cells with different degrees of malignancy affect chemoresistance. Moreover, it is not

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clear how matrices prepared with cells derived from different tumor tissue sources affect the chemoresistance of tumor cell-derived matrices, although the tissue sources of the cells for the preparation of matrices are important for the regulation of normal cell functions on cell-derived matrices [10,11].

In addition to the cell sources for matrix preparation, the initial substrates used for matrix preparation could affect the ability of cell-derived matrices because ECM deposition is strongly affected by protein adsorption, which is regulated by the surface properties of the initial substrates [12]. We have previously reported that blood compatible polymers of poly (2-methoxyethyl acrylate) (PMEA) and poly (tetrahydrofurfuryl acrylate) (PTHFA) enable tumor cells to attach, although protein adsorption characteristics, such as the amount of protein adsorption and adsorption-induced conformational change, are different [13]. This difference in protein adsorption characteristics can lead the cells to form ECMs of different compositions. Thus, it may be possible to increase the chemoresistance of cancer cells on cell-derived matrices prepared on these polymer substrates with different characteristics of protein adsorption.

In this study, we compared the chemoresistance of cancer cells on tumor cell-derived matrices prepared with cells obtained from different tumor tissues at various degrees of malignancy to determine the effects of the malignancy and tissue sources of the cells on the preparation of the matrices. In addition, we compared the chemoresistance on cell-derived matrices prepared with substrates that exhibit different protein adsorption characteristics to determine the effects of the characteristics of the initial substrates.

2. Materials and methods

2.1. Preparation of cell culture substrates

PMEA and PTHFA were synthesized according to previous reports [14,15]. PMEA and PTHFA were dissolved in methanol and methanol/chloroform (5:1) at a concentration of 0.2 wt%, respectively. Twelve microliters of each polymer solution was added to a 96-well tissue culture polystyrene (TCPS) plate, which was subsequently air-dried for seven days. The prepared substrates were sterilized via exposure to UV light on a clean bench for 2 h.

2.2. Cell culture for ECM formation

The human invasive breast cancer cell line, MDA-MB-231; the human mammary gland benign cell line, MCF-10A; the human invasive colon cancer cell line, HT-29; the human non-invasive colon cancer cell line, SW480; and the normal colonic epithelial cell line, CCD-841-CoN, were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The human non-invasive breast cancer cell line, MCF-7, was obtained from the Health Science Research Resources Bank (Osaka, Japan). MDA-MB-231 and MCF-10A cells were seeded on bare TCPS, PMEA-, and PTHFA-coated substrates at densities of 1×10^4 cells/cm^2 and were cultured for 2 weeks in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Ham) (1:1) (DMEM/F-12, Gibco, Carlsbad, CA) containing 10% fetal bovine serum (FBS, Equitech-Bio, Kerrville, TX). MCF-7, HT-29, SW480, and CCD-841-CoN cells were seeded at densities of 3×10^4 cells/cm^2 and were cultured for 2 weeks in DMEM/F-12 containing 10% FBS.

2.3. Preparation of tumor cell-derived matrices

Six types of tumor cell-derived matrices were prepared as new culture substrates using a method similar to that previously reported [9]. Briefly, after being cultured for two weeks on bare TCPS, PMEA, and PTHFA substrates in DMEM/F-12 containing 10% FBS, the cellular components were removed from the matrices through incubation with phosphate-buffered saline (PBS) containing 0.5% Triton X-100 and 20 mM of NH_4OH for 5 min at 37 °C. Subsequently, the samples were treated with 100 μg/ml of DNase I (Roche Applied Science, Penzberg, Germany) and 100 μg/ml of RNase A (Nacalai Tesque, Kyoto, Japan) for 1 h at 37 °C. After the cellular components were removed, the matrices were treated with 0.1% glutaraldehyde in PBS for 6 h at 4 °C to stabilize the matrices and were subsequently treated with 0.1 M glycine in PBS.

2.4. Chemoresistance test against 5-fluorouracil (5-FU)

The cancer cells were seeded on the tumor cell-derived matrices and bare TCPS at a density of 3×10^4 cells/cm^2. After one day of culture in DMEM/F-12 containing 10% FBS, the media were changed to DMEM/F-12 containing 10% FBS supplemented with 5-fluorouracil (5-FU, Sigma) at the indicated concentrations. After an additional three days of culture, the viable cells were evaluated using the WST-8 assay. The data were expressed as the percentage of the number of cells exhibiting growth inhibition relative to the number of the cells cultured without anti-cancer drugs.

2.5. Statistical analysis

All data were presented as the mean ± SD ($n = 3$). All statistical analyses were performed using Microsoft Excel 2010. Significant differences were detected using Student's t test. P-values < 0.05 were considered to be statistically significant.

3. Results

3.1. Effects of the tissue source of the cells used for the preparation of matrices on 5-FU resistance

We examined whether the tissue source of the cells used for the preparation matrices affected the chemoresistance against 5-FU. The percentages of growth inhibition of MDA-MB-231 and HT-29 cells were evaluated on tumor cell-derived matrices using the WST-8 assay (Fig. 1). Chemoresistance of MDA-MB-231 cells against 5-FU increased only on MDA-MB-231 cell-derived matrices (Fig. 1A). Similar chemoresistance of MDA-MB-231 cells against 5-FU was observed on other matrices. Furthermore, we also examined the chemoresistance of HT-29 cells against 5-FU to examine whether MDA-MB-231 cell-derived matrices specifically increased the chemoresistance. Chemoresistance of HT-29 cells against 5-FU increased on only HT-29 cell-derived matrices and not on MDA-MB-231 cell-derived matrices. These results indicated that only matrices derived from cells that had a similar tissue source as the target cells exhibited an increase in the chemoresistance against 5-FU in the chemoresistance assay.

3.2. Malignancy effects of the cells used for the preparation of matrices on 5-FU resistance

The chemoresistance of the invasive cancer cell lines of MDA-MB-231 and HT-29 increased on the corresponding cell-derived matrices. However, it is still not clear whether the chemoresistance of the target cells at lower malignancy increases on matrices derived from cells that were similar to the target cells in the chemoresistance assay (*i.e.*, the effect of the malignancy of the cells used to prepare the matrices is unknown). Next, we examined the chemoresistance of the non-invasive cancer cell lines of MDF-7 and SW480, which exhibit lower malignancy than MDA-MB-231 and HT-29 on matrices prepared with cells derived from the

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