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Cell culture of human gingival fibroblasts, oral cancer cells and mesothelioma cells with serum-free media, STK1 and STK2

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Abstract. The majority of cells are cultured with Dulbecco's modified Eagle's medium (DMEM) or RPMI supplemented with fetal bovine serum (FBS), which contains numerous factors, including cytokines, nutrients and unknown growth factors. These factors may affect cell growth, apoptosis and differentiation. The serum-free medium, STK2, has been previously reported as suitable for the cell culture of human mesenchymal stem cells. However, how STK1 or STK2 affect the cell proliferation of normal and cancer cells remains unknown. The present study examined the growth of the human gingival fibroblast (HGF-1) cell-line and the HSC-3, CA9-22 and MSTO cancer cell-lines, cultured with STK1 and STK2. STK1 increased the cell proliferation of HGF-1 compared to DMEM by assessment with the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, whereas STK1 and STK2 markedly inhibited the cell proliferation of HSC-3 and MSTO. The cell proliferation rate of CA9-22 cultured with STK1 or STK2 for 96 h was ~2-fold higher than the rate for 24 h culture. The shape of the HSC-3 cells was also found to have changed to round when cultured with STK2. These results indicate that STK1 increased the cell proliferation of HGF-1 compared to DMEM, whereas the proliferation of HSC-3 and MSTO was inhibited by STK1 and STK2. Thus, STK1 and STK2 had different affects on the cell growth of HGF-1, CA9-22, HSC-3 and MSTO.

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Introduction

Fetal bovine or calf serum (FBS or FCS, respectively) includes unspecified amounts of growth factors, which may modify the immune response and cell differentiation (1-4). In addition, cell growth and differentiation may be affected in cell culture, depending on the amount of FBS or FCS present.

A previous study demonstrated that FBS inhibited cell growth and increased apoptosis (4). Compared to FBS-supplemented media, serum-free media does not differ between individual batches, and thus, it may be suitable for the cell culture performance. There are several types of serum-free media, specific media is suitable for stem cells and others are acceptable for primary cell culture (5,6). These serum-free media do not affect cell growth and differentiation in cell culture. STK2 is a serum-free medium that has been developed for use with human mesenchymal stem cells (hMSC) (7). STK2 had a minimal effect on the gene expression and morphology of hMSC after 50 days of culture, whereas Dulbecco's modified Eagle's medium (DMEM) with 10% FBS significantly changed the expression of ~1,000 genes and the cell morphology (7). Although STK2 is suitable for hMSC culture, it remains unknown whether STK2 can promote the growth of normal and cancer cells.

CA9-22 and HSC-3 are human oral squamous cell carcinoma cell lines that are often used for *in vitro* assays. CA9-22 highly expresses epidermal growth factor receptor (EGFR), whereas HSC-3 is prone to lymph node metastasis (8,9). Compared to HSC-3, it appears likely that histone deacetylase 2 is highly expressed in CA9-22 (10). MSTO is a human malignant mesothelioma cell-line that highly expresses EGFR (11). These cells have been cultured previously using general medium with 10% FBS (12-14). The present study aimed to examine how STK1 or STK2 affected the cell growth of human gingival fibroblasts (HGF-1), CA9-22, HSC-3 and MSTO by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. STK1 increased the proliferation of HGF-1 compared to DMEM, whereas STK1 and STK2 inhibited the proliferation of HSC-3 and MSTO. CA9-22 was able to grow with STK, but the proliferation rate of CA9-22 cultured with STK was lower than with DMEM. These results indicate that STK affects cell

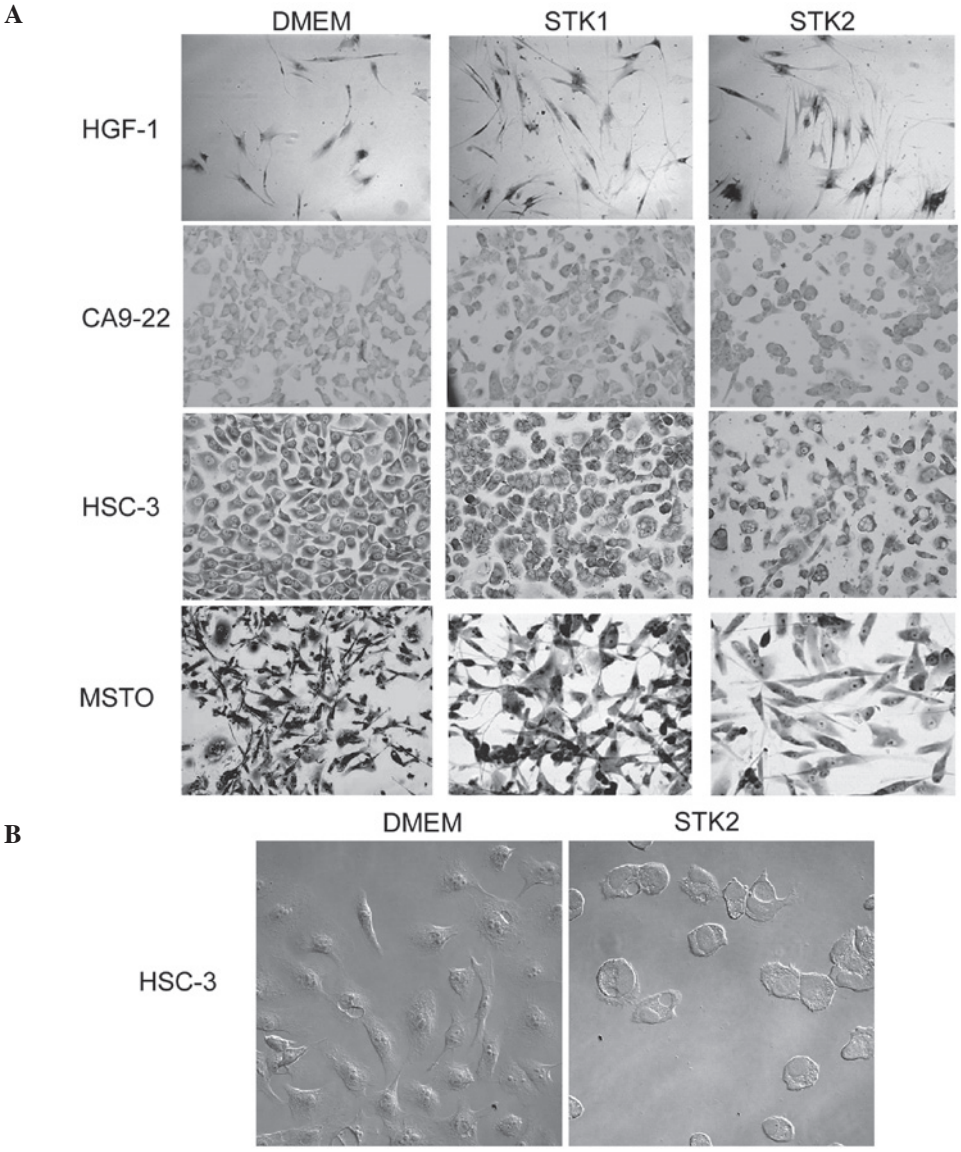

Figure 1. Dulbecco's modified Eagle's medium (DMEM), STK1 and STK2 show differential effects on cell proliferation. (A) Human gingival fibroblast (HGF-1), CA9-22, HSC-3 or MSTO cells were cultured in chamber slides. The culture medium was changed to DMEM, STK1, or STK2 after 24 h and the cells were cultured for an additional 96 h before fixation. (B) HSC-3 cells were cultured with DMEM or STK2 and were fixed. Images were captured in bright-field mode.

growth in the different cell lines, HGF-1, CA9-22, HSC-3 and MSTO.

Materials and methods

Cell culture. HGF-1 and MSTO mesothelioma cells were obtained from the American Type Culture Collection (Manassas, VA, USA). CA9-22 and HSC-3 human oral cancer cell lines were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). These cells were cultured with DMEM-high glucose (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% FBS, STK1 or STK2 serum-free media (DS Pharma Biomedical Co., Ltd., Osaka, Japan) at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. Images of the cells were captured using a Zeiss microscope (Carl Zeiss, Oberkochen, Germany).

Cell viability assay. The cell viability assay was performed using the MTS assay. The cells were seeded in 96-well plates. After culturing for 24 h, the culture medium was changed to DMEM, STK1 or STK2, and the culture was continued for 96 h. CellTiter 96® AQ_{ueous} One Solution reagent (Promega Corporation, Madison, WI, USA) was added to each well and the cells were incubated at 37˚C for 1 h. The absorbance (OD_{490} nm) was measured using a 96-well plate reader of iMark Microplate Absorbance Reader (Bio-Rad, Tokyo, Japan).

Statistical analysis. The data are provided as mean ± standard deviation. Student's t-test was used for statistical analysis ($P<0.05$).

Results

STK inhibits the cell proliferation of HSC-3 and MSTO. The affect of STK1 or STK2 was examined with regards to the cell growth of normal and cancer cells by the MTS assay. HGF-1, CA9-22, HSC-3 and MSTO cells were cultured with DMEM, STK1, or STK2 for 24-96 h. The cell proliferation of

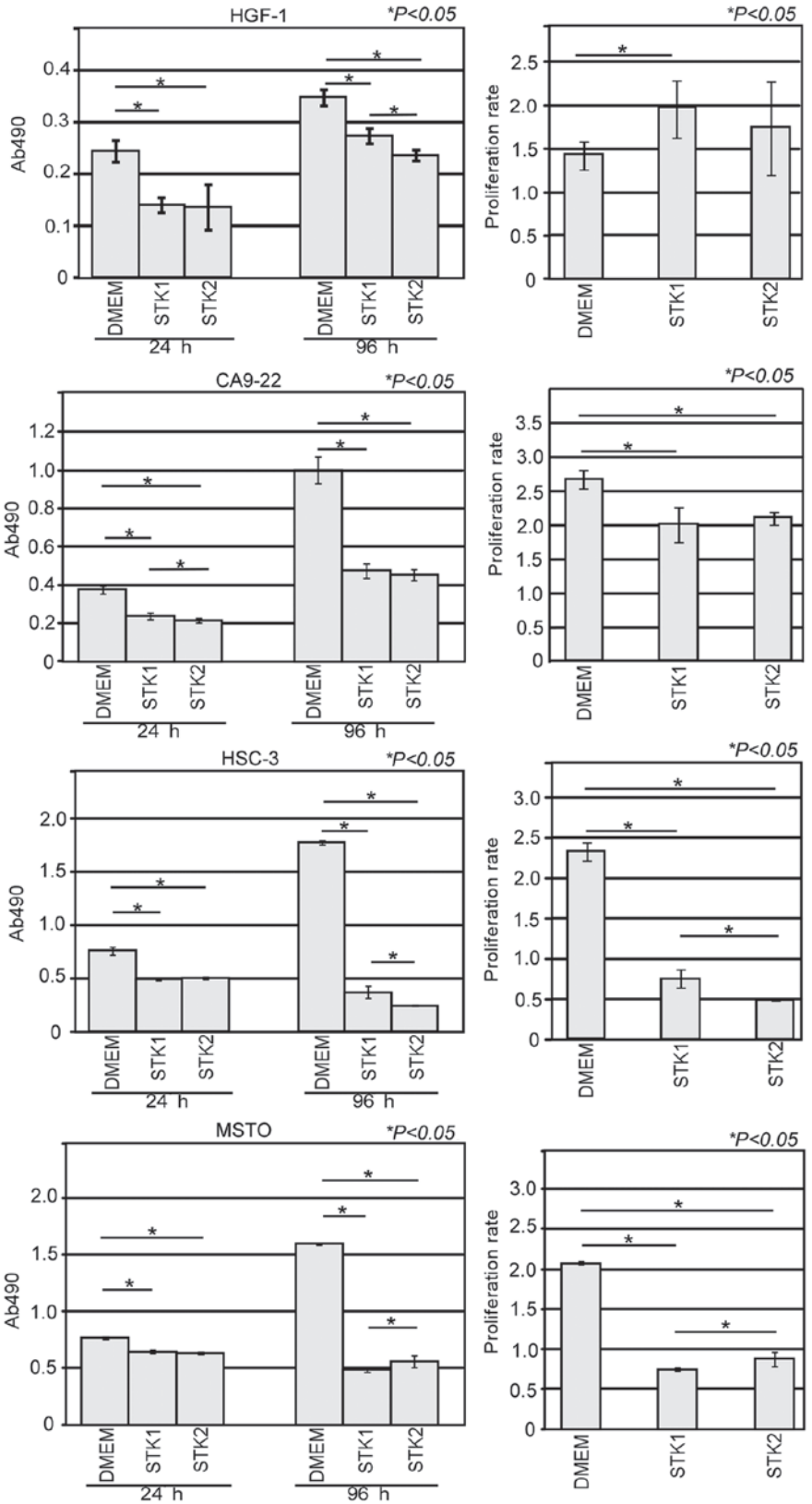

Figure 2. Quantification of human gingival fibroblast (HGF-1), CA9-22, HSC-3 and MSTO cell proliferation at 24 and 96 h when cultured with Dulbecco's modified Eagle's medium (DMEM), STK1 and STK2. The cells were cultured and the MTS-assay was performed. The left panel shows the raw cell proliferation data at 24 and 96 h of cell culture; the right panel shows the cell proliferation rate as determined by Ab490 nm at 96 h, divided by the value at 24 h.

HSC-3 at 96 h after the medium was changed was markedly inhibited by STK1 and STK2. For MSTO, some floating cells were observed when cultured with STK1 (Fig. 1A). STK2 also affected the cell morphology of HSC-3, which changed to a round shape (Fig. 1B). The cell proliferation rates of HGF-1 cultured with DMEM, STK1 and STK2 were 1.4, 2.0

and 1.7-fold, respectively (Fig. 2). There was a significant increase in the cell proliferation rate between DMEM and STK1 ($P<0.05$), whereas there was no significant difference between DMEM and STK2 or STK1 and STK2. The cell proliferation rates of CA9-22 cultured with DMEM, STK1 and STK2 were 2.7, 2.0 and 2.1-fold, respectively. There was a significant decrease in the cell proliferation rate between DMEM and STK1, or DMEM and STK2 ($P<0.05$), whereas there was no significant difference in the cell proliferation rate between STK1 and STK2. The cell proliferation rates of HSC-3 cultured with DMEM, STK1 and STK2 were 2.3, 0.8 and 0.5-fold, respectively. There were significant decreases in the cell proliferation rates between DMEM and STK1, between DMEM and STK2 ($P<0.05$), and between STK1 and STK2 ($P<0.05$). The cell proliferation rates of MSTO cultured with DMEM, STK1 and STK2 were 2.1, 0.7 and 0.9-fold, respectively. There were significant decreases in the cell proliferation rates between DMEM and STK1, and DMEM and STK2 ($P<0.05$), and there was also a significant increase in the cell proliferation rate between STK1 and STK2 ($P<0.05$).

Discussion

Previously, certain studies have performed cell culture without FBS or FCS for pre-clinical data or embryonic stem cells, as the serum affects the cell culture performance (15-17). STK1 and STK2 are serum-free media with unclear roles in the culture of normal and cancer cells. In the present study, the STK media were demonstrated to inhibit the cell proliferation of HSC-3 and MSTO, whereas they increased the cell proliferation of HGF-1 and CA9-22. When cultured with DMEM, these cancer cells grew well compared to STK1 and STK2. There are several possible explanations as to why STK media inhibited HSC-3 and MSTO cell proliferation. First, STK may have induced apoptosis or cell cycle arrest in HSC-3 and MSTO cells, as STK2 was found to induce a round shape in HSC-3. In addition, MTSO cultured with STK1 gave rise to a number of floating or detached cells. Whether this was due to apoptosis or detachment, and the explanation for why STK1 did not induce as many floating cells with HSC-3, could not be elucidated. STK1 may also possibly affect MSTO cell adhesion molecules, including β-catenin, claudins and E-cadherin (18,19).

CA9-22 and MSTO highly express EGFR, which may give rise to different proliferation rates between these two cell types when cultured with STK. Additionally, other factors may regulate cell proliferation, including the clock genes, *PER1* and *PER3*, which are closely associated with cisplatin-induced apoptosis in CA9-22 (8,20). PER1 is highly expressed in CA9-22, whereas it is weakly expressed in HGF-1. Deleted in esophageal cancer 1 (DEC1) regulates cell proliferation under a serum-free condition (21). These molecules are closely associated with the regulation of apoptosis, cell cycle arrest and cell proliferation (20,22,23). Therefore, it can be speculated that PER1, PER3 and DEC1 may be involved in the regulation of cell proliferation by STK. These studies indicate that HSC-3 and MSTO cells may require more nutritional components than HGF-1 and CA9-22. Therefore, future studies should clarify how STK affects apoptosis and the cell cycle.

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