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Effects of L-fucose supplementation on the viability of cancer cell lines

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Abstract

Background: Fucose is a deoxyhexose sugar. While the biological roles of L-fucose remain unclear, the sugar is known to accelerate the malignant potential of cancer cells. Therefore, this study aimed to evaluate the viability pattern of human cancer and normal cell lines treated with fucose. **Methods**: The human gingival fibroblast (HGF-1), colorectal adenocarcinoma (HT-29) and skin malignant melanoma (A375) cell lines were cultured and treated with fucose at three concentrations of 1, 5, and 10 mg/ml. Cell viability was then measured using (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay. The data were analyzed using Statistical Package for the Social Sciences software. **Results**: The percentage of HGF-1 cell viability showed a rapid decline after day 1 of treatment. HT-29 and A375 were capable of surviving treatment with high fucose concentrations. The data were highly significant at $p < 0.001$. **Conclusion**: Whereas a high concentration of fucose is toxic to the HGF-1 cell line, the HT-29 and A375 cell lines could potentially adapt to this condition. Down- or upregulation of certain molecules that could induce or inhibit cell death may explain such adaptation. Further testing of up- and downregulated molecules should be conducted in future work.

Keywords: adenocarcinoma, cancer, cell line, fucose, glycosylation, melanoma

Introduction

Cancer presents multiple risk factors. Hyperglycemia has been reported to raise the prevalence and mortality of certain cancers, such as breast, liver, bladder, pancreatic, colorectal, and endometrial cancers.[1] Hyperglycemia could promote the proliferation, invasion, and migration of cells and induce apoptotic resistance.[1] This condition is also known to be a factor for the development of cancer among patients with diabetes, especially type 2 diabetes.[1,2] Fucose is a natural deoxyhexose sugar with a structure similar to that of glucose except for its lack of a hydroxyl group on carbon 6.[3] Mammalian cells utilize fucose in the L-enantiomer form, although other deoxyhexoses are used in the D-enantiomer form. L-Fucose is incorporated onto glycoproteins during the synthesis of *N*- and *O*-linked glycans in mammalian cells.[4] Fucosylated glycans perform several functions, such as inflammatory response regulation, signal transduction, cell growth, transcription, and adhesion. For example, cell–cell interactions can be partially modulated by the presence of L-fucose-specific lectin-like adhesion molecules on the cell surface. Fucosylation of cell membrane receptors and proteins, including epidermal growth factor receptor (EGFR), TGFβ, Notch, E-cadherin, integrins, and selectin ligands, has also been reported to influence ligand binding, dimerization, and signaling capacities.[3] Activation of EGFR tyrosine kinases is a key factor for lung cancer progression.[5] Increased L-fucose in serum has been detected in breast, oral, head, neck, liver, ovarian, and prostate cancers.[3]

Addition of glycans to glycoproteins is known as glycosylation. Glycosylation is the major posttranslational modification of proteins contributing to malignant transformation and metastasis.[6,7] Fucose is a basic constituent of oligosaccharides and associated with cancer and inflammation. Increases in fucose level may lead to altered or unique glycoconjugates.[8] Fucose is one of the essential components of the Lewis ligands, selectin family that play the important role for cell adhesion. For instance, during inflammation, E- and P-selectins appear on activated endothelial cells to interact with leukocytes through sialyl-Lewis (x) and sialyl-Lewis (a) antigens.[9] These selectins represent typical tumor-associated carbohydrate antigens that have been reported to be responsible for the adhesion of human cancer cells to the endothelium.[10] Fucosylation produces fucosylated markers, and the upregulation of these markers could promote canceration. Previous study reported that key enzymes, such as alpha1,2-

fucosyltransferases (e.g., FUC1 and FUC2), are highly expressed in malignant tissues, for example, breast cancer.[11]

Several checkpoints are available in the cell cycle, and these checkpoints are crucial in preventing abnormal or cancer cells from replicating. When a checkpoint detects the abnormality of a cell, signals for repair and apoptosis are initiated. Supplementation of the cell culture medium plays a pivotal role in modulating available checkpoints. For instance, high L-fucose concentrations cause fucosylation, which subsequently produces molecules that could prevent apoptosis. Normal and cancer cells similarly require nutrients to replicate. Treatment with high concentrations of fucose is expected to cause either up- or downregulation of molecules that are important to cell replication. In addition, when treated with high concentrations of fucose, normal and cancer cell lines could show similar or different effects in terms of cell viability. Therefore, the cell viability patterns of normal and cancer cell lines treated with high concentrations of fucose should be investigated to explore possible treatments for cancer. Information related to these patterns could be used to improve cancer therapy in the near future. Herein, this study aimed to investigate the viability patterns of human cancer and normal cell lines treated with three different concentrations of fucose.

Methods

Selection and preparation of cancer and normal cell lines. Three cell lines were used in this study, namely, the human colorectal adenocarcinoma (HT-29, ATCC HTB-38, USA) cell line, the human malignant melanoma (A375, ATCC CRL-1619, USA) cell line, and the human gingival fibroblast (HGF-1, ATCC CRL-2014, USA) cell line. In this work, the colorectal adenocarcinoma and malignant melanoma cell lines were used to examine the effects of fucose treatment on the internal and external parts of the body, respectively. The human gingival fibroblast cell line was selected because cells of the mouth are the first site in which metabolism takes place.

Selection of monosaccharides. The monosaccharide used in this study, L-fucose, was procured from Nacalai Tesque Inc. (Kyoto, Japan) in powder form.

Optimization of concentration and treatment period. The methods used to optimize the concentration and treatment period for in vitro stability studies were adapted and modified from a previous study.[12] Fucose was diluted properly to three different concentration of 1, 5, and 10 mg/ml. The monosaccharide was dissolved completely in DMEM to ensure no possible physical effect toward the cell lines. The control for this study was composed of DMEM containing only 10% fetal bovine serum (FBS) and 1% penicillin + streptomycin solution. Four incubation periods of 1, 3, 5, and 10 days were applied as a treatment parameter to study the effect of fucose on the cell viability of the tested cell lines.

Cell culture technique. The HT-29, A375, and HGF-1 cells were thawed and revived in a 25 ml culture flask containing basal medium and incubated overnight at the optimum temperature of 37 ℃ with 5% CO_2 and 90% humidity. The medium was changed after 3 days of incubation to ensure sufficient nutrients for the cells to develop and grow. Cell proliferation activity was observed daily until approximately 80% confluence was achieved. The cell cultures were then subjectively observed by comparing occupied and unoccupied areas. Upon reaching confluence, the cells were dissociated using dissociation media and then seeded.

Viability test by MTT assay. MTT assay was conducted to assess the metabolic activity of the cells. Prior to the cell viability test, the cells were counted using a hemocytometer by adopting the method from previous study.[13] Approximately 1000 cells were aliquoted into different 12-well plates containing DMEM that had been added with 10% FBS, 1% antibiotics, and fucose. Plates with fucose were incubated for 1, 3, 5, and 10 days.

The treated cell lines were washed with phosphate buffer saline. Then, 20 µl of 0.5 mg/ml MTT solution was added to each well, and the plates were incubated for at least 2 hours. The MTT solution was then removed, and the wells were added with 200 µl of dimethyl sulfoxide to solubilize the formazan crystals. Absorbance was measured using a spectrophotometer at 450 nm. Because all cell viability procedures were considered light-sensitive processes, the plates were wrapped with aluminum foil.

Analysis of data. Data were analyzed by using Statistical Package for the Social Sciences software. All treatment groups were compared with the control group, and values are expressed as standard error mean ± standard deviation. Significant differences between treatment groups and the control were determined using one-way ANOVA. A $p \leq 0.05$ was considered to indicate statistical significance.

Results

Table 1 reveals the cell viability of the normal HGF-1 cell line after treatment with fucose. Comparison of the cell viability patterns of the control and different treatment groups reveals remarkable differences. This result indicates that the cell viability pattern of control group is likely similar to that of normal cell growth. All of the treatment groups showed a drastic decline in cell viability after day 3 of treatment, a slight increase on

day 5, and another decrease on day 10. While the effect of 5 mg/ml fucose on cell viability on day 10 seems to be less extensive than that of 10 mg/ml fucose, the observed viability patterns are similar.

Table 2 illustrates the viability of A375 cells after treatment with fucose. The control and 1 mg/ml fucose treatment groups showed similar patterns of cell viability, which means 1 mg/ml fucose does not affect cell viability. The control and 1 mg/ml fucose treatment groups showed continuous increases in cell viability from day 1 to day 3. Cell viability declined from day 5 to day 10. Treatment of A375 cells with 5 and 10 mg/ml fucose revealed no increase for the cell viability which the cells continue to decline from day 1 until day 10. Unlike HGF-1 cells, these cancer cells were able to withstand the effects of high fucose concentrations; thus, the cell viability of this line did not decline on days 5 and 10 in comparison with that of the HGF-1 line. Fucose treatment resulted in concentration-dependent decreases in cell viability. However, cell viability after treatment with 5 mg/ml fucose on day 10 appeared to be higher than that observed after treatment with 1 mg/ml fucose.

Table 1. Percentage of HGF cell viability after treatment with 1, 5, and 10 mg/ml fucose ($p < 0.001$)

Day	Untreated (%)	1 mg/ml (%)	5 mg/ml (%)	10 mg/ml (%)
Day 1	100.00	98.21	98.92	99.48
Day 3	97.62	5.50	5.19	5.26
Day 5	111.27	6.50	5.96	6.64
Day 10	87.80	2.10	1.43	2.05

Table 2. Percentage of A375 cell viability after treatment with 1, 5, and 10 mg/ml fucose ($p < 0.001$)

Day	Untreated (%)	1 mg/ml (%)	5 mg/ml (%)	10 mg/ml (%)
Day 1	100.00	105.70	106.41	113.78
Day 3	138.22	102.20	96.54	92.36
Day 5	49.02	40.03	49.54	41.07
Day 10	22.31	3.97	11.25	10.06

Table 3. Percentage of HT-29 cell viability after treatment with 1, 5, and 10 mg/ml fucose ($p < 0.001$)

Day	Untreated (%)	1 mg/ml (%)	5 mg/ml (%)	10 mg/ml (%)
Day 1	100.00	102.06	102.76	109.63
Day 3	159.85	130.67	98.77	85.68
Day 5	139.71	105.87	89.85	76.27
Day 10	69.57	46.80	59.87	53.17

Table 4. Comparison of HGF–Fucose, A375–Fucose, and HT-29–Fucose using One-way ANOVA

Fucose	Sum of Squares	Df	Mean Square	F	*p*
HGF– Fucose					
Between Groups	0.065	15	0.004	38.982	< 0.001
Within Groups	0.009	80	< 0.001		
Total	0.074	95			
A375 – Fucose					
Between Groups	46.485	15	3.099	32.083	< 0.001
Within Groups	42.888	444	0.097		
Total	89.373	459			
HT-29 – Fucose					
Between Groups	44.186	15	2.946	306.272	< 0.001
Within Groups	4.463	464	0.010		
Total	48.649	479			

Table 3 shows the cell viability patterns of HT-29 cells after treatment with fucose. The cell viability of the control group indicated an increment from day 1 to day 3. Cell viability then declined from day 5 to 10. By contrast, the cell viability of all treatment groups declined from day 1 to day 10. In addition, on day 1, cell viability increased as the treatment concentration increased. An increase in cell viability was also observed on days 5 and 10 after treatment with 5 mg/ml fucose. By comparison, cell viability decreased after treatment with 10 mg/ml fucose. Table 4 shows that the results of ANOVA are highly significant with $p < 0.001$.

Discussion

Cellular growth may be categorized into four important phases. A previous study explained the phases of cellular growth.[14] The first phase is known as the lag phase. During this phase, cellular adaptation to the new environment takes place, new enzymes are synthesized, and a slight increase in cell mass and volume occurs, but the cell number does not increase. The second phase is the exponential phase, during which balanced growth, that is, all cell components grow at a similar rate, occurs. In the phase, the cells have adjusted to their new environment and replicate exponentially. The growth rate is independent of the nutrient concentration because nutrients are in excess in this phase. The third phase, the stationary phase, begins when the net growth rate is equal to zero. Finally, the declining or death phase is characterized by a decrease in the living cell population over time because of a lack of nutrients and increase in the amount of toxic metabolic by-products.

Excess fucose in the medium would affect the cell growth of normal and cancer cell lines. According to the findings in this study, excess fucose presents a level of toxicity that causes normal human gingival fibroblasts to die. This toxic effect could be due to the oxidative stress effect. The oxidation process of fucose yields toxic metabolic by-products. Yorek and Dunlap reported that L-fucose causes the generation of reactive oxygen species (ROS) and activation of NF-kB after cellular processing.[15] Excess ROS has been reported to cause DNA damage, which subsequently leads to cell death.[16] NF-kB plays an important role in causing apoptosis, which is also known programmed cell death.[17] Therefore, normal cells die under high concentrations of L-fucose.

The cell viability patterns of the cancer cells reveal that these cells could adapt to a new environment. Moreover, the results indicate that cancer cells could survive treatment with excess monosaccharides, which may be due to the disruption of the apoptotic pathway. For instance, a previous study reported that mutations or downregulation of molecules involved in the Fas receptor–Fas ligand (FasR–FasL) apoptotic pathway are well-known mechanisms exploited by cancer cells to escape apoptosis.[7] Elevation of ROS in cancer cells is a hallmark of cancer cell progression. Recent studies have demonstrated that cancer cells are highly adaptive to elevated levels of ROS by activating antioxidant pathways. ROS play a vital role in cancer development, including initiation, promotion, and progression.[16] Increased intracellular ROS levels may activate oncogenes and oncogenic signals, including constitutively active mutant Ras, Bcr-Abl, and c-Myc, all of which are involved in cell proliferation, inactivation of tumor suppressor genes, angiogenesis, and mitochondrial dysfunction.[16]

Glycosylation is an enzyme-directed site-specific process that links saccharides to produce glycans, which, in turn, are attached to proteins, lipids, or other organic molecules.[18] Glycosylation is one of the most important posttranslational modifications and related to many different diseases.[19] It is involved in numerous essential biological processes, such as cell proliferation, differentiation, migration, cell–cell integrity and recognition, and immune modulation.[19] As mentioned earlier, fucosylated glycans have functions in inflammatory response regulation, signal transduction, cell growth, transcription, and adhesion. For example, cell–cell interactions can be partially modulated by the presence of L-fucose-specific lectin-like adhesion molecules on the cell surface.[3] Breast cancer tissues overexpress fucosylated glycans, such as sialyl-Lewis X/A, and α-1,3/4-fucosyltransferases to promote disease progression and metastasis.[20]

Although the biological roles of L-fucose remain unclear, the monosaccharide is known to accelerate the malignant potential of cancer cells.[21] Previous research on human breast cancer suggested that α-L-fucose is not a bystander molecule but a pathophysiological effector.[22] Indeed, α-L-fucose has been proposed to be an important component of the malignant and metastatic phenotypes of human breast cancer.[22] Other studies conducted using cell lines derived from several adenocarcinomas, certain melanomas, and some leukemias and lymphomas reveal drastic differences in the glycan expression of neoplastic cells in comparison with that of normal cells.[22] In 2010, glycans were shown to be intrinsically important in the pathobiology of most common human malignancies.[23] A review paper reported the role of fucosylated ligands in human breast cancer, particularly as expressed in CD44 variants.[22] Cancer cells directly take up L-fucose and secrete fucoproteins.[21] Fucoproteins are glycoproteins containing fucose sugar units as one of their carbohydrates.[18] Changes in fucoprotein could help determine the cancer diagnosis and prognosis.[24] An increase in enzymes involved in fucosylation would result in increased levels of fucosylated proteins.[25]

Physiologically, serum concentrations of L-fucose are generally low in normal persons but high in cancer patients.[26,27] An earlier study reported that fucose levels are higher in serum and tissue of the cancer patients.[28] Elevation of L-fucose levels in the serum and body fluids may be attributed to the release of glycoproteins from the tissue as a result of cell destruction or the local synthesis and secretion of glycoproteins by tumor cells.[21] However, some investigators believe that an increase in serum L-fucose levels reflects tissue proliferation rather than tissue destruction.[3,29] Fucosylated haptoglobin, which appears in the serum of some cancer patients, may be a promising biomarker for the prognosis of colorectal cancer.[30]

Early detection of cancer is very important to achieve treatment efficacy. Loud and Murphy reported that cancer treatment is most effective when the disease is detected at an early stage prior to the onset of symptoms.[31] A previous study reported that the 5-year survival rate for patients with breast cancer is nearly 99% if the cancer is detected at the early stages.[20] However, if the tumor has metastasized, the survival rate drastically decreases to 25%.[20,32] Knowledge of the effects of fucose and fucoproteins could be harnessed to provide effective cancer treatment. For instance, fucosylation inhibitors could be applied to treat cancer. An earlier study reported that the use of 2-fluorofucose (2-FF) inhibits the adhesion of a human primary breast cancer cell line to E-selectin under physiologic flow conditions and reduces the migration ability and proliferation rate of this line.[20] Another study showed that the cell proliferation and integrin-mediated cell migration of a liver cancer cell line are significantly suppressed by treatment with 2FF.[33]

Conclusion

According to the cell viability patterns observed in this study, treatment with high concentrations of fucose is toxic to the HGF-1 cell line. By contrast, the HT-29 and A375 cell lines appear to be able to adapt to high fucose concentrations. The ability of these cancer cells to survive could be due to the downregulation of molecules involved in the apoptotic pathway. For instance, molecules involved in the FasR–FasL apoptotic pathway are well-known mechanisms exploited by cancer cells to escape apoptosis. Hyperglycemia induces chemoresistance, which allows cells to proliferate and undergo progression.[34] Therefore, future studies should be conducted to investigate the molecules potentially involved in allowing cancer cells to survive in the presence of high fucose. Future advances in technology may see fucose become a target for anti-cancer therapy via nanoparticles or micellar formation.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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