RESEARCH ARTICLE

Viability, Migration Rate, and mRNA Expression of GLUT5, GLUT7, GLUT11 in WiDr Colorectal Cancer Cell Line

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Abstract

BACKGROUND: Insufficient glucose levels in colorectal cancer (CRC) patients leads to a condition where fructose might become an alternative source for cells proliferation, but the role of fructose or fructose-glucose combinations in development of CRC has not been elucidated well. In this study, the effect of fructose-glucose variations on viability, migration, and glucose transporter (GLUT)5, GLUT7, GLUT11 mRNA expressions in WiDr CRC cell line were examined.

METHODS: Cells were treated with varying ratios of fructose-glucose (F100%; F75%:G25%; F50%:G50%; F25%:G75%; G100%; F: Fructose, G: Glucose). Untreated cells (F0:G0) were used as cell control. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used for cell viability test, scratch assay was used to examine the cell migration, and quantitative polymerase chain reaction (qPCR) was performed to examine mRNA expressions. Data were analyzed using one-way analysis of variance (ANOVA) and followed with Tukey's post-hoc test, with $p<0.05$ consideres as significant.

RESULTS: Fructose-glucose combinations and glucose 100% significantly increased the cell viability compared to control ($p<0.05$). All treatment groups showed a significant increase in cell migration compared to control ($p=0.000$). Only GLUT7 and GLUT11 expressions in the G100% group were significantly different compared to the control ($p=0.000$). GLUT7 and GLUT11 expressions were also significantly different in F100% and F50%:G50% treatments compared to G100% ($p=0.000$).

CONCLUSION: Taken together, fructose might play important role in cell migration. However, in cell viability, combination with glucose could increase fructose's effect. Fructose might not affect the mRNA expressions of GLUT5, GLUT7 and GLUT11.

KEYWORDS: GLUT5, GLUT7, GLUT11, fructose transporter, colorectal cancer, WiDr

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Introduction

Abnormal changes in glucose metabolism provide the necessary substrate for cancer cell proliferation and division, thereby causing tumor growth and metastatic development.(1) Patients with colorectal cancer (CRC) have a higher average daily intake of glucose and fructose, so fructose is also often associated with tumorigenesis and an increased risk of CRC.(2) Fructose metabolism can be

utilized by tumor cells as an alternative energy source to maintain proliferation and increase metastasis in CRC.(3,4) The aberrant metabolism of glucose and its role in cancer metabolism has been well investigated, but the role of fructose in cancer needs to be investigated in more detail. (5) Glucose transporter (GLUT) facilitates the transport of sugars and has a role in the absorption of glucose and fructose (6), but there is still limited knowledge of other GLUTs besides GLUT1-5.(7) GLUT5 is not only associated with cancer growth and energy expenditure but also with the migration of cancer cells caused by changes in metabolism.(8)

GLUT7 and GLUT11 have high sequence similarity to GLUT5, and can facilitate the transport of fructose and glucose (7,9,10), but research on this GLUT is still lacking concerning cancer development. WiDr cells are used in this research because WiDr cells are a model of colorectal cancer cells that express mutant PIK3CA and p53 genes. (11,12) PIK3CA mutations can increase proliferation and glycolysis processes both in normal and low glucose conditions, while p53 gene mutations can increase metastasis in colorectal cancer.(13-15) There is a limited number of studies regarding the effect of fructose or the combination of fructose-glucose on CRC and its effect on GLUT, especially GLUT5, GLUT7, and GLUT11, so this study was conducted to evaluate how much fructose and the combination of fructose-glucose play a role in the growth, migration, and expression of fructose transporters in CRC.

Methods

Cell Culture and Reagents

Colorectal cancer WiDr cell lines were cultured and maintained in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium (Cat. #11965092, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) (Cat. #F0804, Sigma-Aldrich/Merck, Darmstadt, Germany), 1% Amphotericin-B (Cat. #15290018, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Cat. #P4333, Sigma-Aldrich/Merck). The culture system was maintained at 37°C in a humidified atmosphere containing 5% CO_2 until 80% confluent.

The cell was treated in DMEM without glucose, L-Glutamin, phenol red (Cat. #A144300, Thermo Fisher Scientific), supplemented with 10 % fetal bovine serum (FBS) (Cat. #F0804, Sigma-Aldrich/Merck), 2% Glutamax (Cat. #35050061, Thermo Fisher Scientific), 1% Amphotericin-B (Cat. #15290018, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Cat. #P4333, Sigma-Aldrich/Merck). Fructose (Cat. #F3510, Sigma-Aldrich/Merck) was dissolved in distilled water into 4500 mg/L and then filtered through a 0.22 µm syringe filter. Glucose solution (Cat. #A249400, Thermo Fisher Scientific) was dissolved in distilled water into 4500 mg/L.

MTT Assay for Cell Viability

WiDr cells were seeded on plate 96 at a density of $5x10^3$ cells/well with treatment concentration series (F100%; F75%:G25%; F50%:G50%; F25%:G75%; G100%; F: Fructose, G: Glucose). Untreated cells (F0:G0) were used as cell control. Well plate with the cells was incubated at 37°C in a humidified atmosphere containing 5% CO_2. Cell viability was determined by the formazan formed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma-Aldrich). MTT solution was added at 0, 24, 48, 72, and 96 hours. MTT solution was added to each well and incubated for 4 hours. The reaction was stopped by adding sodium dodecyl sulfate (SDS) then incubated in the dark at room temperature overnight and then read using a microplate reader (620 nm). All experiments samples were performed in triplicate.

Cell Migration (Scratch Assay)

Cells were seeded on plate 24 at a density of $5x10^4$ cells/well. After 80% confluency, cells were scratched using a 200 µL sterile pipette tip. The wells were rinsed with phosphate-buffered saline (PBS) until completely cleaned, so no cells stuck to the scratches and no cells floated. The picture was taken of the stretch results in five fields of view (0-hour observation). The PBS was removed from the well by pipetting slowly and replaced with treatment concentration series (F100%; F75%:G25%; F50%:G50%; F25%:G75%; G100; F: Fructose, G: Glucose). Untreated cells (F0:G0) were used as cell control. All experiments samples were performed in triplicate. Observations and documentation were carried out at 0, 18, 24, and 42 hours in five fields view using the same inverted microscope (Olympus, Tokyo, Japan), the same magnification, the same camera, and settings. ImageJ software (National Institutes of Health, Bethesda, MY, USA) is used to calculate the closure at each observation time. The migration rate is expressed as a percentage of closure.

Cell Treatment for mRNA Expression

Cells that were 80% confluent from the flask were seeded in a 6-well culture plate with a density of $0.3x10^6$ in DMEM

medium with varying concentrations of fructose:glucose (F100%; F50%:G50%; G100%; F: Fructose, G: Glucose). Cells were incubated for 72 hours. Untreated cells (F0:G0) were used as cell control, and each treatment was performed in duplicate.

Extraction

After 72 hours of treatment, total RNA was extracted using TRIzol® (Cat. #15596026, Invitrogen, Carlsbad, CA, USA) according to manufacturer's instructions. Total RNA from each concentration was checked for its RNA concentration using a spectrophotometer (260 nm) and synthesized to cDNA using iSCriptTM cDNA Synthesis Kit (Cat. #1708890, Bio-Rad Laboratories, Hercules, CA, USA).

Quantitative Real-Time PCR (qPCR)

The qPCR was performed using the SensiFAST™ SYBR ® No-ROX Kit (Cat. #BIO-98020, Bioline Reagents, London, UK). The reverse-transcribed cDNA from each sample after 72 hours of treatment was PCR-amplified with primers based on the GLUT5, GLUT7, and GLUT11 gene sequences (Table 1) with the following reaction condition profile: 15 min at 95°C, 40 cycles of 30 sec at 94°C, 30 s at 53°C and 30 s at 72°C. The relative gene expression was calculated using Livak Method (16), and normalized with β-actin as the housekeeping gene. All the primers were purchased from PT. Genetika Science Indonesia (Tangerang, Indonesia).

Statistical Analysis

All the data were presented as mean±SD and visualized by using GraphPad Prism software ver. 9.00 for Windows (GraphPad Software, San Diego, CA, USA). The data were analyzed with significance tests by one-way analysis of variance (ANOVA) and continued with Tukey post-hoc test by using SPSS (IBM Corporation, Armonk, NY, USA), and $p<0.05$ was considered as the significant value of difference.

Results

The Combination of Fructose-Glucose Increases the Viability of WiDr

All treatment groups, either with fructose, glucose, or a combination of both, increased cell viability at all observation times. The combination of F50%:G50% showed the highest viability value and F100% showed the lowest viability value after 96 hours of treatment. After 96 hours of treatment, the F75%:G25%; F50%:G50%; F25%:G75% and G100% groups had a significance $p<0.05$ compared to the control, but F100% was not significantly different compared to the control. There was no significant difference in viability between fructose, glucose, and combination fructose-glucose treatment. CRC WiDr cell line had nearly the same viability when induced with glucose or a combination of fructose-glucose, as shown in Figure 1 and Table 2.

Fructose Induction Level Affects Cell Migration in WiDr

Cell migration rate on all treatments had a significant $p=0.000$ compared to the control at 48 hours of observation. A comparison of cell migration rates in all treatments is shown in Figure 2. The highest cell migration rate is found in G100%, however, the addition of fructose and glucose with the same levels or the addition of fructose with lower levels will increase the cell migration rate compared to fructose only. The fructose-glucose combination with higher fructose levels showed a lower cell migration rate when compared to fructose only.

Fructose Transporter mRNA Expression

There was no significant difference in the initial test for the value of viability and migration rate between one treatment and another except for the control, including the fructose-glucose combination of F75%:G25%, F50%:G50%, F25%:G75% ratio. Based on these results, we only took the

Table 1. Primers for GLUT5, GLUT7, GLUT11, and β-Actin used in this study.

Gene	Primer Forward (5'-3')	Primer Reverse (5'-3')
GLUT5	CCTTTGGGTCATCCTTCCA	ACAGACCACAGCAACGTCAA
GLUT7	TCGGTGCCTACAGTTTCATC	AATGCGGTTTATCTCCACAA
GLUT11	CGTGATGGGACAGGTGGT	GCTTTCAGGGAGCAGAGG
β- actin	CGCGAGTACAACCTTCTTGC	ATACCCACCATCACACCCTGG

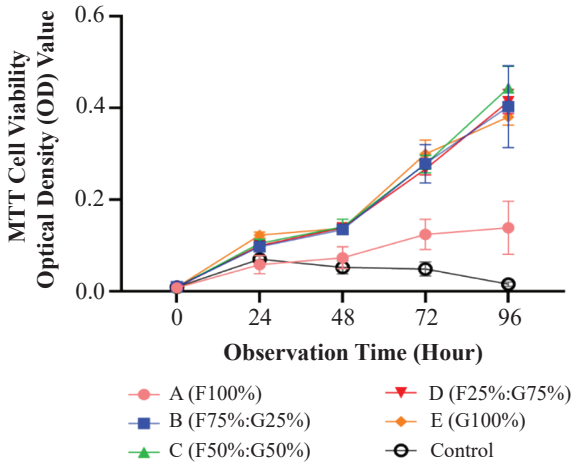

Figure 1. WiDr cell viability in various treatments. Data were normalized by medium control values. All data were represented as mean±SD.

ratio of F100%, G100%, and the F50:G50% for the mRNA expression test. The qRT-PCR data showed that GLUT5, GLUT7, and GLUT11 were expressed in all treatments, but only GLUT7 and GLUT11 in the G100% treatment showed significant differences compared to the control (p=0.000 for each). In GLUT7 and GLUT11 mRNA expressions, G100% treatment also showed significant differences compared to the F100% and F50%:G50% treatment (p=0.000 for each) (Figure 3).

Discussion

One of the causes of colorectal cancer is changes in glucose metabolism (17), but fructose metabolism in causing disease in mammals is not fully understood (18). The effect of fructose or fructose-glucose combinations on the growth of colorectal cancer cells was obtained by comparing cell viability and migration rate in several treatment groups. This study uses a concentration ratio of F50%:G50% based on the general composition range of sucrose and High Fructose Corn Syrup (HFCS) as sweeteners that are often encountered in daily use which contain almost the same ratio of fructose and glucose.(19) With this standard, we then varied the concentration above and below this ratio in stages (F75%:G25%; F25%:G75%; F100%; G100%).

Although glucose is still used as the main energy source in the WiDr cancer cell line, the addition of fructose at certain concentrations in the combination medium affects cell growth and migration. These results are in line with *in vivo* tests in mouse where a combination of fructose and glucose diets can increase tumorigenesis. (20) Through the ketohexokinase-aldolase-B pathway, fructose promotes colon cancer metastasis to the liver. (16) Fructose metabolism can downregulate mitochondrial respiratory function and increase aerobic glycolysis which can help metastasis so that fructose is often associated as an alternative energy source for cancer cells.(21,22)

The uptake of glucose are associated by GLUT expression.(23) While, fructose metabolism is facilitated by GLUT5, GLUT7, and GLUT11.(10,24) GLUT7 is relevant for high affinity for fructose and glucose, but its expression in cancer has not been determined.(9) Expression of GLUT7 was increased by fructose (25), but there is a single binding site on GLUT7 that recognizes glucose and fructose which can inhibit each other's transport effectiveness (26). The existence of a conserved isoleucine-containing motif that is present in GLUT7 may also be involved in the signaling pathway in fructose transport.(26)

GLUT11 is one of the least studied GLUTs.(27) GLUT11 can facilitate fructose and glucose.(28) D-glucose up-regulated mRNA levels in GLUT11 (29), this is following the results of our study where the highest GLUT11 expression as well as the value of its viability was found at a concentration of 100% glucose. GLUT11 has a low-affinity value for both glucose and fructose so its ability to

Table 2. Treatment groups' cell viability compared with controls.

Treatment Group	Mean Difference	95% Confidence Interval		*p*-value
		Lower Bound	Upper Bound	
F100%	0.04	-0.09	0.17	0.942
F75%:G25%	0.15	0.14	0.28	0.021*
F50%:G50%	0.16	0.02	0.29	0.011*
F25%:G75%	0.14	0.01	0.28	0.020*
G100%	0.15	0.02	0.28	0.015*

Tested with one-way ANOVA continued with Tukey post-hoc. *p<0.05 is considered as significant.

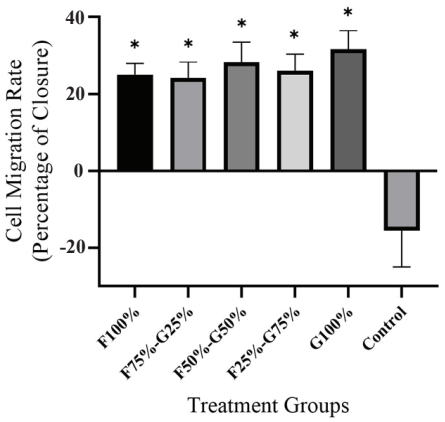

Figure 2. Quantitative analysis of cell migration rate in CRC WiDr cell line at various treatment. Migration rates are expressed by calculating the difference in closure observed at 0 to 48 hours. All experiments samples were performed in triplicate and represented as mean±SD. *p=0.000 compared to control (untreated cells).

transport glucose-fructose is not as good as other GLUTs (30), and fructose is also known to inhibit glucose transport activity in GLUT11.(31) This may cause differences in GLUT11 expression in F100% and F50%:G50% versus G100%.

Various literature states that GLUT5 is only specific as a fructose transporter (7,32), but the data showed that besides being detected in the fructose treatment, the fructose-glucose combination, GLUT5 was also expressed in the 100% glucose treatment, although its expression was not significant compared to controls. Cells treated with normal serum showed glucose to be a better inducer than fructose for GLUT5 expression.(33) Glucose derivatives can also induce GLUT5 expression by activating ChREBP as a downstream of GLUT5.(34) Several other components affect cell growth. Apart from fructose and glucose which are transported by GLUT, there is also the amino acid compound l-alanyl-l-glutamine as a source of amino acids in cell growth media. L-alanyl-L-glutamine, a dipeptide compound containing glutamine, has a role in gut function. (35) These compounds can also increase other chemical compounds' absorption, stimulate cellular proliferation, and inhibit apoptosis caused by cell damage (36), so that the optical density and GLUT values have different trends even though they are in the same group. Further research is needed on other fructose and glucose concentration ratios and their influence on fructose transporter expression.

Conclusion

Cell viability was significantly affected by treatment of fructose and glucose at ratios of F75%:G25%, F50%:G50%, F25%:G75% and G100%. Treatment of F100%, F75%:G25%, F50%:G50%, F25%:G75%, and G100% had a significant effect on cell migration. In addition, GLUT7 and GLUT11 mRNA expressions were significantly increased under treatment of G100% while GLUT5 mRNA expression was also increased under treatment of G100%, although not significant. Taken together, fructose might play important role in cell migration. However, in cell viability, combination with glucose could increase fructose's effect. Fructose might not affect the mRNA expressions of GLUT5, GLUT7 and GLUT11.

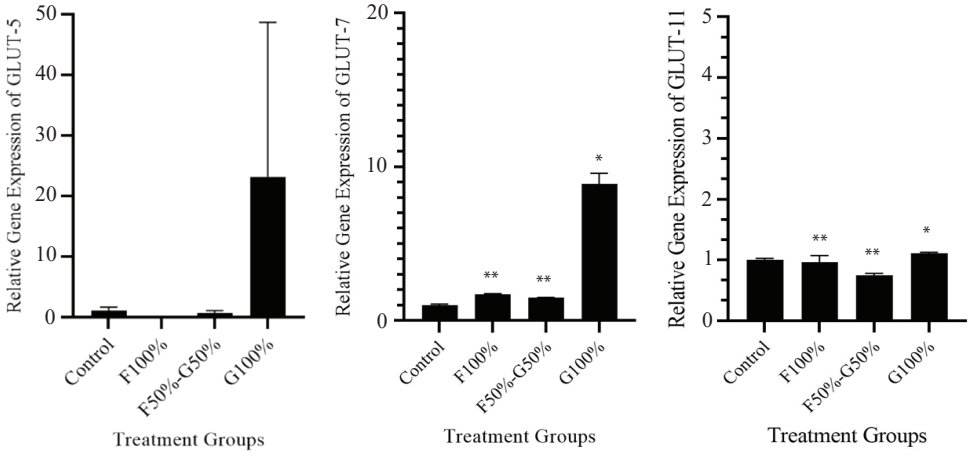

Figure 3. Relative gene expression of GLUT5, GLUT7, GLUT11 in various treatment. The mRNA relative expression levels of colorectal cells line WiDr after being supplemented with fructose–glucose in various concentrations. GLUT expressions were analyzed using the Livak Method and normalized using β-actin. All data were represented as mean±SD. *p=0.000 compared to control (untreated cells). **p=0.000 compared to G100%.

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Authors Contribution

NH, MH, and IY were involved in the design of the research concept and result interpretation. NH collected the data, analyzed the data, and drafted the manuscript. MH and IY were involved in the design manuscript, visualizations, and manuscript composition. RN, II, and LH were involved in giving critical revisions for important content in this research. HA was responsible for instrument research.

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