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Articles

Features of Glucose Utilization and Oxygen Consumption by Mcf-7 Breast Cancer Cells Depending On in Vitro Culture Conditions

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Abstract

This study aimed to show the possibility of regulating the metabolic capabilities of cancer cells *in vitro* by changing the medium composition, using the MSF-7 breast cancer cell line as an example. The relevance of the work is due to the solid necessity for constantly improve cell lines for testing new antitumor drugs and obtaining new information about the chemistry of cancer.

The assessment of glucose oxidation type by measuring the production of lactate and polarographic determination of oxygen consumption under normal conditions and the influence of Oligomycin A, 2,4 Dinitrophenol, or Rotenone action were basic methods of this study. An excess of glucose or removing glutamine was created as medium composition changes. A culture of Vero non-tumor cells was applied as a comparative model.

As a result, the lactate production by MCF-7 tumor cell culture is significantly higher than the same by Vero culture, which is consistent with the Warburg effect. Excess glucose led to decreased lactate production in MCF-7 culture by almost 10 %, but it was not observed for Vero culture. The glutamine decrease in the culture medium does not significantly affect the change in lactate production for both Vero cells and MCF-7 cells. In the culture of tumor cells, excess glucose and lack of glutamine during daily incubation did not statistically affect respiration, while these changes were accompanied by a decrease in oxygen consumption by cells by 10 % and 15 %, respectively, in the culture of Vero cells.

The obtained data can be used to control the cultivation system and verify metabolic changes due to testing new antitumor agents *in vitro*.

Keywords: breast cancer, cell lines, MSF-7 cells, Vero cells, cancer cell metabolism, glucose utilization, oxygen consumption.

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1. Introduction

Malignant neoplasms are one of the leading causes of death from diseases. According to the World Health Organization, about 8 million people die from cancer every year in the world. Breast cancer is one of the most dangerous types of cancer among women. The five-year survival rate of women diagnosed with metastatic breast cancer is slightly less than 30 % (Bray et al., 2018, DeSantis et al., 2016).

Even though many approaches are used for cancer treatment, including surgical and radiation ones, chemotherapy is one of the primary treatments at all stages of breast cancer in combined regimens. For metastatic cancer, chemotherapy becomes the method of choosing (O'Shaughnessy et al., 2012).

Since the disease is polymorphic, and chemotherapeutic agents necessarily pass the *in vitro* testing stage, we need to identify and study different human breast cancer cell lines constantly.

The most relevant is assessing the metabolic phenotype of tumor cells due to the widespread prevalence of oncological diseases and the emergence of new, more accurate, and powerful equipment (Clegg et al., 2020, Dai et al., 2017).

The tumor transformation of cells is a complex multi-stage process that leads to a change in many fundamental principles of metabolism. It is widely known that tumor cells acquire the ability to unlimited proliferation (1); growth autonomy and insensitivity to growth factors (2); metastasis progression (3); genome instability and metabolic reprogramming (4); and apoptosis avoidance (5). The metabolic reprogramming in a tumor cell affects all vital pathways as the exchange of proteins, fats, and carbohydrates, allowing transformed cells to adapt to new conditions of existence (Butler et al., 2020, El-Sahli, Wang, 2020, Kang et al., 2018).

Glucose is the primary source of energy in cells. Under normal conditions, aerobic glycolysis dominates in human cells, thanks to which glucose utilization formed 90 % ATP. During anaerobic glycolysis, pyruvate is converted into lactate, some of which is then converted into acetyl-CoA in the mitochondria and then is oxidized to CO_2 (Nelson, Cox, 2014).

Increased glucose uptake is typical for tumor cells. As a result, the increased lactate formation manifested in its early stages of tumor transformation even with high oxygen content in the medium. This change is called the Warburg effect, implemented at the expense of high intensity of anaerobic glycolysis. The active use of glutamine and the synthesis of higher fatty acids are other essential features of cancer cell metabolism (Gentric et al., 2017, Van der Heiden, DeBerardinis 2017).

These metabolic differences between normal and cancer cells can serve as a biochemical basis for developing new antitumor drugs. Inhibition of glycolysis, changes in glutamine metabolism, and fatty acid synthesis are three possible approaches to antitumor metabolic therapy (Bhardwaj, He, 2020, Park et al., 2020, Pattni et al., 2017). In practice, a combination of traditional chemotherapy with drugs that affect a particular stage of cancer cell metabolism is the most effective method of tumor therapy.

Based on the above, we have devoted this study to evaluate the features of glucose utilization and energy metabolism by tumor cells *in vitro* culture under when metabolic control by changing the culture medium composition.

2. Material and methods

Characteristics of tumor cell cultures

MCF-7 culture is one of the primary cell lines of human breast cancer, so we used it in this study. Currently, MCF-7 is the most popular line for studying the cytotoxicity of antitumor agents and for the molecular features of breast cancer. The MCF-7 cells have an epithelial-like structure, express estrogen receptors, and synthesize estradiol, making them optimal targets for studying receptors and chemotherapy. MCF-7 lines form monolayer cultures with a survival rate of up to 95 % and a confluence of up to 100 % due to the preservation of reproductive ability (Alexandrova et al., 2019, Comsa et al., 2015).

The transferable Vero culture is the renal epithelial cells of the African green monkey. It is widely used to cultivate viruses, detect Escherichia coli toxins, and host cells for eukaryotic parasites. The Vero cells have a fibroblast-like structure. They form monolayer cultures on media with a survival rate of 70-90 % and up to 100 % (Osada et al., 2014). In our work, we used the Vero cell line as a variant of non-tumor phenotypes for comparison with the behavior of the MCF-7 line.

Applied kits and reagents

Table 1 presents the list and purpose of the used kits and reagents by their application in this study.

Table 1. Characteristics of the kits and reagents applied in the study

Kit/Reagent	Application	Abbreviation
Medium MEM without glutamine (PanEco)	Cell culture	MEM
Medium DMEM without glutamine (PanEco)		DMEM
Fetal calf serum (PanEco)		FCS
2 mM solution of L-glutamine (PanEco)		Glutamine
6 mM solution of D-Glucose (PanEco)		Glucose
A mixture of antibiotics (streptomycin + penicillin, PanEco)		AB
Trypsin 0.25% solution (PanEco)	Manipulations with cells	Trypsin
Versen's solution (PanEco)		VS
Hanks' solution (PanEco)		HS
Buffer, pH 7.5, tris-HCl 50 mM, p-chlorophenol 6.0 mM (Sigma)		TrisB
Trypan blue solution (PanEco)	Determination of viability	TB
70 % ethyl alcohol	Antiseptics	Eth
3.3 mM lactic acid solution (Sigma)	Lactate measuring	Lactat
Kit for lactate measuring of Olvex Diagnosticum		Olvex kit
Polarography medium (1 mM EDTA + 1.2 mm $MgCl_2$ + 1 M taurine + 5 mm KH2PO4 + 20 mM HEPES buffer + 100 mM sucrose, pH 7.4)	Polarography	PM
10 µM of Oligomycin A solution (Sigma-Aldrich)		Oligomycin
100 µM of 2,4-dinitrophenol solution (Sigma)		DNP
10 µM of Rotenone solution (Sigma-Aldrich)		Rotenone
3 M solution of Potassium chloride (Sigma-Aldrich)		KCl
3 % Hydrogen peroxide solution		H_2O_2

Cell culture technique

After thawing the cells, we washed them twice in HS solution, using centrifugation for 5 min at 500g for precipitation. The culture vials with a capacity of 10 ml contained a standard complete medium of the composition MEM/DMEM + Glutamine + FCS + AB. A MEM was used for the MCF-7 cell culture in all experiments, and DMEM was used for the Vero culture. The reproduction providing the necessary number of cells took place in the thermostat at a temperature of 37 °C and in the presence of 5 % CO_2 in the gas phase. If necessary, we replaced the nutrient medium using as a criterion the level of cell viability in the trypan blue test and its morphology visualized using Olympus inverted microscope.

Determination of glucose oxidation pathway in cell cultures

The MCF-7 and Vero cells grew for 24 hours in a standard medium (1), in a medium with a glucose excess (2), and a medium without glutamine (3). The number of cells in each well was 2×10^5.

We planted the pre-prepared MCF-7 and Vero cell cultures on 24-well plates at the rate of 100 µL/well using a standard complete medium of the composition MEM/DMEM + Glutamine + FCS + AB. Cultivation took place until ~ 95 % confluence in each well. Then the medium was replaced in each well to ensure the protocol of the experiment. Three variations of the MEM medium and three variations of the DMEM medium were necessary for this procedure.

The first variations made in eight wells on each plate were control ones, and they contained a standard complete medium. The subsequent eight wells contained glucose in addition to the standard complete medium. The third variant of medium did not contain glutamine in eight wells

on each tablet. After adding 2 mL of the appropriate medium to each well, the plates were cultured for 24 hours. Then the culture liquid was taken from the wells, centrifuged for 3 minutes at 500 g, and lactate was determined using a commercial Olvex Diagnosticum kit following the manufacturer's protocol. The optical density of the experimental and calibration samples was measured using an Olvex Diagnosticum spectrophotometer against a blank sample at a wavelength of 505 nm. The lactic acid concentration was expressed as

$$C = 3.3\ E_e/E_c \ \text{mM},$$

where E_e and E_c denote the optical densities of the experimental and calibration samples, respectively.

To provide the next stage of the study, we removed cells from the wells maintaining the marking adopted at the first stage and carried out the following experimental method.

Determination of oxygen uptake by cells

The measurement was performed using the cell metabolism analyzer Seahorse XFe24 (Agilent Technologies) by polarography.

Initially, the polarography medium, solutions of oligomycin, DNP, rotenone, KCl, and distilled water were incubated in a water thermostat at a temperature of 33 °C for 10 min. Next, 1 mL of a polarography medium containing 2×10^6 MCF-7/Vero cells were placed in a polarographic chamber. The respiration rate was sequentially recorded with this composition of the chamber, after the introduction of 100 µL of oligomycin as an ATP synthase inhibitor, after the introduction of 100 µL of rotenone to inhibit the I complex, and after the introduction of 100 µL of DNF. Cellular respiration in each case was recorded for 3 min.

Statistical procedures

Statistical analysis was performed using the software package Statistica 12.0 (StatSoft Inc., USA). Distribution in the samples was expressed as mean ± SEM. Statistical significance in differences between results was evaluated using Welch's t-test as two-tailed Student *t*-test with unequal variances. The differences were considered statistically significant when the *p*-value was less than 0.05.

3. Results

Lactate production under various cultivation conditions

The study showed that the lactate production by MCF-7 culture due to cultivation in a standard medium was significantly higher than by Vero cells (Table 2, Figure 1).

Table 2. Lactate concentration in MCF-7 and Vero cell cultures, mM/L

Cell line	Standard medium	Excess glucose	Without glutamine
MCF-7	9.55 ± 0.13	8.58 ± 0.28 #	9.63 ± 0.25
Vero	7.21 ± 0.11 *	7.03 ± 0.10 *	7.18 ± 0.07 *

Here and in Table 2, the * sign denotes significant differences in the values of the indicator between cell lines (p less than 0.05), the # sign denotes significant differences in the values of the indicator between the lines in an intact environment and its change.

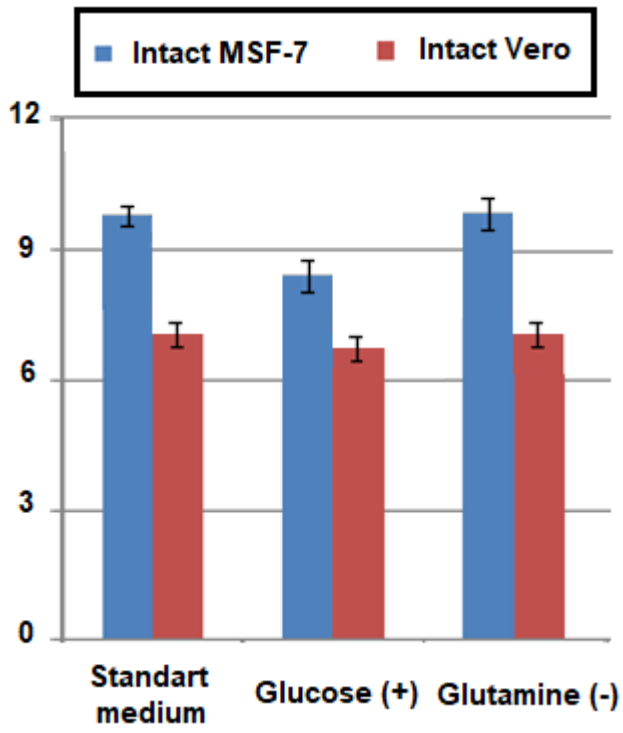

Fig. 1. Lactate production (mM/L of the final concentration) in the MCF-7 and Vero cell cultures under various cultivation conditions

Cultivation of MCF-7 in an environment with a glucose content of more than 1 g/L led to a decrease in lactate production by 9 %, while Vero cells practically did not change this production. The absence of glutamine in the culture medium did not significantly affect the lactate production for both MCF-7 and Vero cells.

Oxygen consumption under various cultivation conditions

Comparison of MCF-7 and Vero cultures showed a reduced O_2 consumption in MCF-7 culture, consistent with the ideas of a predominantly anaerobic energy flow in tumor cells (Table 3, Figure 2).

The measuring of cellular respiration after H_2O_2 treatment (negative control) gave the following results. The O_2 consumption decreased by 55.34% in comparison with the basic respiration data. The addition of oligomycin, DNF, or rotenone decreased O_2 consumption by 63.6 %, 53.0 %, and 42.67 %, respectively. After the damage by H_2O_2, the differences between the cultures lost their statistical significance, proving the validity of the applied method.

Table 3. Respiration rates of MCF-7 and Vero cultures under various culture conditions (nM/s per 10^6 cells)

Cell line	Standard medium	Excess glucose	Without glutamine
Basic respiration			
MCF-7	11.90 ± 1.08	11.06 ± 1.04	11.30 ± 0.76
Vero	15.34 ± 0.90 *	13.89 ± 1.15 *	13.72 ± 0.97 *
Oligomycin			
MCF-7	7.98 ± 1.12	7.02 ± 1.07	7.50 ± 0.62
Vero	10.54 ± 1.05 *	9.11 ± 0.98	9.16 ± 1.36
DNP			
MCF-7	12.84 ± 0.97	12.70 ± 1.22	13.10 ± 1.02

Vero	16.55 ± 1.05 *	15.34 ± 1.23 *	14.67 ± 1.14
Rotenone			
MCF-7	8.90 ± 0.73	8.11 ± 1.24	8.01 ± 0.97
Vero	9.28 ± 0.83	8.33 ± 0.82	8.39 ± 1.41

The incubation of MCF-7 culture in the medium with increased glucose content was accompanied by a decrease in the respiration level of 1-5 %, which was insignificant. Similar comparisons in the Vero cell culture showed that in an environment with an increased glucose content, the indicators of basic respiration were 9.4 % lower than the control ones. The use of oligomycin, DNF, or rotenone led to the lowering of O_2 consumption by 13.6 %, 7.3 %, and 10.2 %.

Comparing intact MCF-7 cell culture with a culture incubated in the medium with the absence of glutamine revealed the decrease in oxygen concentration by an average of 1-4 % for all tests, which is insignificant. In the Vero cell culture, glutamine deprivation led to a 10.6 % decrease in basic respiration. O_2 consumption decreased by 13.1 %, 11.4 %, and 9.6 % in oligomycin, DNP, and rotenone tests, respectively.

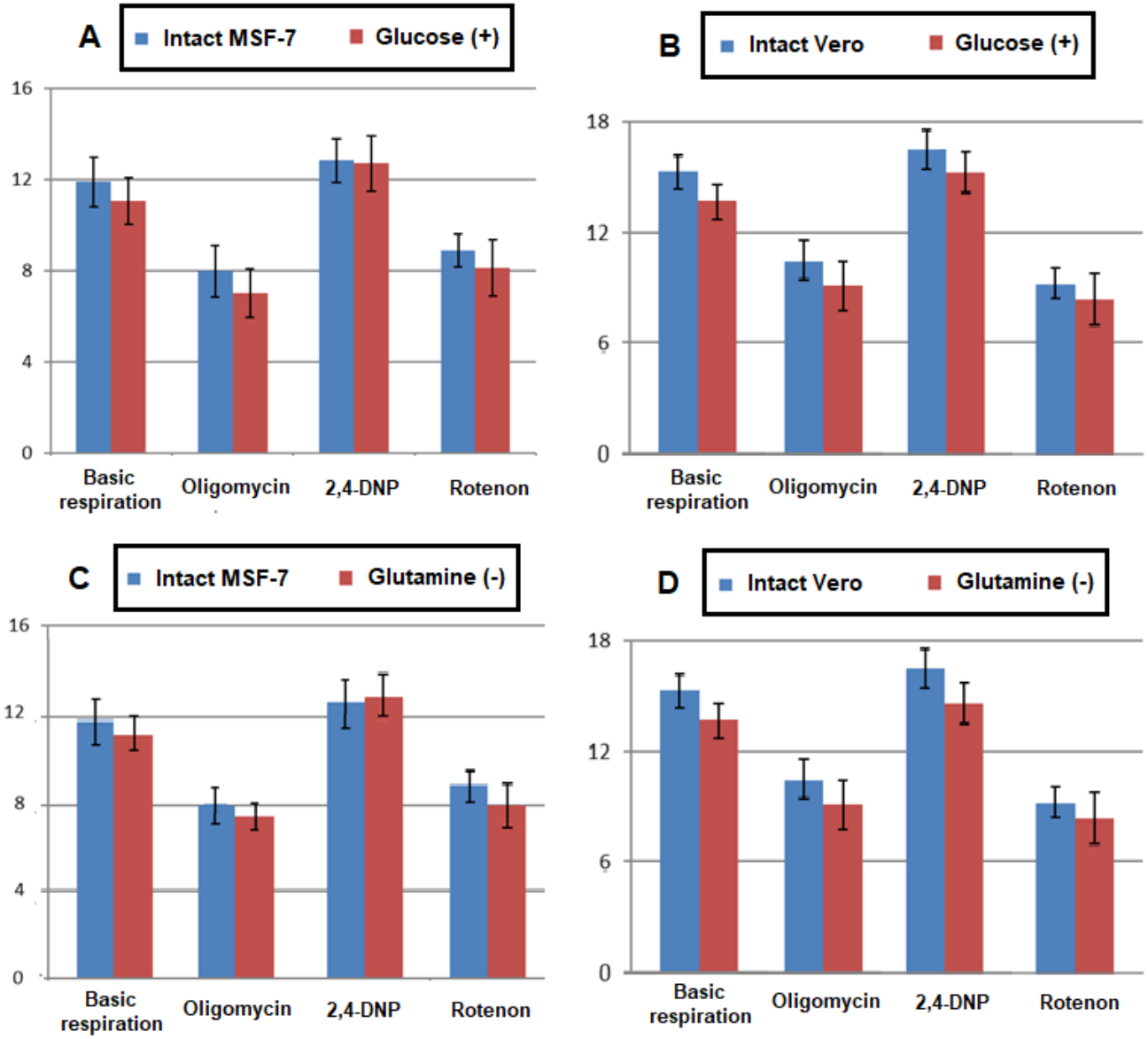

Fig. 2. Average O_2 consumption (nM/s per 10^6 cells) in cell cultures under various culture conditions on polarography data. A. MCF-7 cell culture in medium with high glucose content. B. The same culture in medium without glutamine. C. Vero cell culture in medium with high glucose content. D. The same culture in medium without glutamine

4. Discussion

The number of oncological diseases has increased significantly. Therefore, it is necessary to identify new and study existing tumor cell lines to diagnose and treat this pathology effectively. All these events are closely related to the metabolic status of the cell, so a study of its metabolic pathways can provide the key to understanding the overall functionality of tumor cells (Jose et al., 2011).

Performing the work, we assumed that lactate production in tumor cells would be higher than in non-tumor cells due to the more intensity of metabolism, including the Warburg effect. Secondly, we considered it most likely that the glucose addition will increase the production of lactate, and the glutamine deprivation in the medium will lead to a decrease in the intensity of this process.

The first assumption was confirmed. In our experiments, the MCF-7 cells showed lactate production almost a third greater than Vero cells. The obtained data are consistent with the theory of metabolic reprogramming (the Warburg effect), characterized primarily by a shift in energy supply from mitochondrial oxidative phosphorylation to aerobic glycolysis. This is the most important distinguishing characteristics that cells acquire during tumor transformation. Lactate is a product of cell metabolism, which significantly increases when there is a lack of oxygen. Tumor cells, being in such conditions, actively produce lactate (Katzir et al., 2019).

As for the effects of glucose and glutamine, the production of lactate in the culture of MCF-7 cells did not increase in the first case, but it slightly decreased. In the second case, no changes were observed. In the culture of Vero cells, neither the glucose excess nor the glutamate absence led to a decrease in lactate production. The explanation of the obtained data may be that the glucose excess is already present in the nutrient medium that we took as the initial, of necessary to meet the energy needs of these cells.

Using polarography, we determined the crucial indicators of cellular respiration, which are vital for understanding the overall metabolic status of tumor cells (Traba et al., 2016).

We found that the basic respiration of tumor cells does not change in case of glucose added to the medium or glutamine absence in it, which may indicate that the MCF-7 culture uses internal mechanisms to switch metabolic pathways depending on environmental conditions. The indicator's value in the MCF-7 culture for all tests turned out to be lower than the same in the Vero culture by an average of 10-20 %. In contrast, Vero cells under these conditions significantly reduced the rate of O_2 consumption.

Oligomycin is an inhibitor of ATP synthase. When it is administered in cells, electrons are transferred along the respiratory chain with minimal ATP formation. Naturally, O_2 consumption, in this case, decreases (Salim et al., 2016). DNP partially uncouples oxidative phosphorylation, and therefore there is a compensatory increase in the O_2 consumption by cells. The rotenone application did not accompany differences in O_2 consumption in the cultures of tumor and normal cells. This fact resulted from its possible to completely inhibit complex I function, and the intensity of complex II functioning practically does not differ in tumor and non-tumor cells under these conditions.

The obtained data are consistent with the theoretical provisions explaining the peculiarities of cell metabolism, which show that glutamine can be used in providing cells with substrates to enhance the work of the respiratory chain and ATP synthesis. However, it should be noted that high activity of glycolytic pathway in tumor cells results in expression of glutamine effect in tumor cells less than in non-tumor ones (Zambrano et al., 2019).

Considering the data we obtained, new studies can be organized aimed at studying the mechanisms of rearrangement of the metabolism of tumor cell lines with changes in the concentrations of primary metabolites in the culture medium, for example, the study of inhibitors of the main metabolic pathways using 2-dehydroglucose, 3-brompyruvate, aminotransferases, etc.

5. Conclusion

Our study found that lactate production in the MCF-7 tumor cell culture is significantly higher than in the Vero cell culture, which is in good agreement with the Warburg effect. The glucose excess is accompanied by a decrease in lactate production in the MCF-7 culture by almost 10 %, but not it is not typical for Vero cell culture. The glutamine absence in the culture medium does not significantly affect the change in lactate production for both Vero and MCF-7 cells.

In the culture of MCF-7 cells, glucose excess or glutamine absence during daily incubation did not statistically affect respiration, while in the culture of Vero cells, these changes were accompanied by a decrease in O_2 consumption by 10 % and 15 %, respectively.

The obtained data can be used to control the cultivation system and verify metabolic changes in testing new antitumor agents in vitro.

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