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Proliferation of Cultured Glioma Cells Mediated by Coenzyme Q_{10} under Conditions of Serum Deprivation

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Abstract—The effect of coenzyme Q_{10} on glioma-cell proliferation under serum-deprived conditions has been studied. Our results have shown that the addition of coenzyme Q_{10} into a serum-free culture medium enhances cell viability, stimulates cell growth, restores mitochondrial potential, and increases the quantity of energized mitochondria. It is found that coenzyme Q_{10}-induced glioma-cell proliferation in conditions of serum deficiency is a result of an intracellular reduced glutathione concentration with subsequent activation of protein kinase C, ERK1/2, and phosphoinositol-3-kinase.

Keywords: ubiquinone, glioma, proliferation, redox state, redox regulation

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INTRODUCTION

Coenzyme Q_{10} (ubiquinone) is a component of the mitochondrial electron transport chain. It provides electron transport and protects membrane structures from damage produced by reactive oxygen species (ROSs) (Crane, 2000; Villalba and Navas, 2000). Ubiquinone exerts a therapeutic effect for neurodegenerative and cardiovascular disorders (Littarru and Tiano, 2010). Application of coenzyme Q_{10} as an effective antioxidant for tumor chemotherapy decreased anticancer drug cardiotoxicity (Conklin, 2005; Littarru and Tiano, 2010; Soni, 2015). On the other hand, it has been demonstrated that chemotherapy drugs, such as camptothecin, etoposide, doxorubicin, and methotrexate, while increasing the concentration of coenzyme Q_{10} in tumor cells, maintain their viability (Brea-Calvo et al., 2006). Ubiquinone is considered to be a redox-regulator of cell functions (Crane, 2000) due to its antioxidant and prooxidant properties providing hydrogen-peroxide production.

Tumor growth is accompanied with chaotic vascularization, resulting in unbalanced supply of tumor cells with oxygen and nutrients (Vaupel, 2004). Fast-growing tumors have areas with circulatory disorders and large gradients of nutrient distribution. Microenvironment heterogeneity in cancer cells is a tumor-progression factor and is a reason for their variable sensitivity to anticancer drugs. Tumor-cell response to biologically active compounds varies depending on the cell metabolic microenvironment. Therefore, there is a need to screen various drugs for tumor cells under conditions maximally close to those in various tumor areas.

Under growth-factor deficiency, serum-free culture medium in particular, ubiquinone inhibits apoptosis in tumor cells (López-Lluch et al., 1999; Navas et al., 2002) and enhances BALB/3T3 fibroblast, as well as leukemic cell (K562 and HL-60), proliferation (Gómez-Díaz et al., 2000). It also inhibits sphingomyelin phosphodiesterase (SMPD), also known as neutral sphingomyelinase (EC 3.1.4.12), and prevents ceramide accumulation, which prevents apoptosis development (Martín et al., 2003). Supposedly, SMPD inhibition results from the enzyme interaction with ubiquinone, as well as from ubiquinone-mediated suppression of lipid peroxidation (Navas et al., 2007). Mechanisms of ubiquinone-induced mitogenic signal transmission are unknown.

In order to apply ubiquinone in cancer therapy, it is necessary to know how coenzyme Q_{10} affects tumor-cell functioning depending on their redox-state and physico-chemical parameters of the microenvironment. The goal of this study was to evaluate the impact of coenzyme Q_{10} on glioma-cell proliferation with serum deficiency in the culture medium and clarify the redox-dependant mechanisms of its action. In this study, we use tumor cells grown in the culture medium

Abbreviations: ROS—reactive oxygen species, EGF—epidermal growth factor, FBS—fetal bovine serum, GSH—reduced glutathione, H_2DCF-DA—2,7-dichlorodihydrofluorescein diacetate, MCB—monochlorobimane, PI3K—phosphoinositol-3-kinase, PKC—protein kinase C, S1P—sphingosine-1-phosphate, SMPD—sphingomyelin phosphodiesterase, SphK—sphingosine kinase.

with serum deficiency as a model of tumor growth in areas with nutrient deficiency.

MATERIALS AND METHODS

Reagents and media. Triton X-100, 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA), propidium iodide, monochlorobiman (MCB), DL-buthionin-(S,R)-sulfoximine (BSO), coenzyme Q_{10}, Dulbecco's modified Eagle medium (DMEM), dimethyl sulfoxide (DMSO), dicoumarol, inhibitors PD098059, Sb203580, SP600125, Gö6983, LY294002, JSH (Sigma-Aldrich, United States); fluorescent probe JC-1 (Life Technologies, United States); gentamicin (OAO Belmedpreparations, Belarus); fetal bovine serum (HyClone, United States); NaCl, KCl, $NaH_2PO_4 \cdot H_2O$, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $NaHCO_3$, glucose (Analysis-X, Belarus); buffer solution (mmol/L): 131.0 NaCl, 5.0 KCl, 1.3 $MgSO_4 \cdot 7H_2O$, 1.3 $CaCl_2$, 6.0 glucose, 20 HEPES.

Cells. Rat glioma C6 was obtained from the Culture Collection of the Institute of Epidemiology and Microbiology (Minsk, Belarus). The cells were maintained in DMEM medium with 10% FBS and 10^{-4} g/mL gentamicin at 37°C. The cells were plated at a density of 10^5 cells/mL. On the second day (70–80% of confluence), the medium was exchanged for a medium with 2% FBS and the cells were cultivated for 24 or 72 h with DMSO (not more 0.1%) (control) or various doses of coenzyme Q_{10}. Coenzyme Q_{10} was added as a solution in DMSO.

Cell viability and proliferation. Glioma C6 cell viability and proliferation were assessed (Kato et al., 1999) with propidium iodide (PI) using a CM2200 spectrophotometer (Solar, Belarus). The cells were washed twice with buffer and then had 4×10^{-6} mol/L PI added to them. Fluorescence intensity F_{d1} (wavelengths of excitation and emission were 530 and 645 nm, respectively) was measured in 5 min. The cells were lysed with 0.1% Triton X-100, and fluorescence intensity F_{t1} was registered. Cell viability in each sample V was determined as the ratio of living cells to the total cell number according to the formula

$$V = \frac{(F_{t1} - F_{t2}) - (F_{d1} - F_{d2})}{(F_{t1} - F_{t2})} \times 100\%, \qquad (1)$$

where F_{d2} and F_{t2} are the PI fluorescence intensities in HEPES buffer with and without Triton X-100, respectively. Viability in each sample V was normalized for viability in control V_0.

Proliferative activity N relative to the control N_0 was determined according to the formula

$$N/N_0 = \frac{(F_{t1} - F_{t2}) - (F_{d1} - F_{d2})}{(F_{t10} - F_{t2}) - (F_{d10} - F_{d2})} \times 100\%, \qquad (2)$$

where F_{d10} and F_{t10} are fluorescence intensities F_{d1} and F_{t1} for the control sample.

Hydrogen-peroxide utilization was measured with 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) fluorescence dye (Wrona and Wardmann, 2006) using a CM 2200 spectrophotometer (Solar, Belarus). An amount of 1 μM H_2DCF-DA was added for 30 min at 37°C. The cells were washed with centrifugation at 1500 rpm for 7 min and suspended in HEPES buffer. The cell number in a sample was 10^6/mL. Fluorescence intensity (wavelengths of excitation and emission were 488 and 530 nm, respectively) was registered after the addition of freshly prepared hydrogen peroxide solution (10^{-5} mol/L).

Mitochondria membrane potential (φ) of C6 glioma cells was measured with JC-1 fluorescent dye (Smiley et al., 1991). The cells were cultivated with 1 μM coenzyme Q_{10} for 20 min or 24 h at 37°C. The cell monolayer washed twice with HEPES buffer was incubated with 5 μM JC-1 in HEPES for 15 min at 37°C. Fluorescence intensity was registered at 530 and 590 nm (excitation at 490 nm). The distribution of JC-1 fluorescence intensity in cells was determined with a confocal scanning micro Raman spectrometer (Nanofinder, Lotis, Belarus–Japan) using a blue laser (excitation at 473 nm). The scanning step was 1 μm. Mitochondrial membrane potential was estimated with the ratio of the fluorescence intensity (I) of the dye in the monomer (at 530 nm) and dimer (at 590 nm) forms (I_{590}/I_{530}).

The concentration of intracellular reduced glutathione (GSH) was determined with monochlorobiman (MCB). Glioma cell suspension (10^6/mL) was incubated in the buffer with 10 μM MCB for 30 min at 37°C. The fluorescence intensity of glutathionyl-conjugates MCB (I^{mcb}) was registered at 390 nm (excitation) and 480 nm (emission). The results are presented as the ratio of concentrations in the tested sample ([GSH]) and the control ([GSH]$_0$) one, which equals the ratio of MCB fluorescence intensity for both samples (I^{mcb}/I_0^{mcb}).

Statistical treatment. The average value was determined for independent measurements (more than three). The results are presented as mean values and the product of the standard deviation and the Student coefficient for the appropriate number of measurements (confidence probability $P = 0.95$). The kinetic dependence given in the work is typical for three to five independent experiments.

RESULTS

The effect of coenzyme Q_{10} on the viability and proliferation of glioma cells cultivated for 24 h under standard culture conditions (10% serum) and serum deficiency (2%) is shown in Table 1. It is seen that 1–10 μM coenzyme Q_{10} does not affect the cell viability and proliferation in cultures maintained with 10% serum. Serum deficiency (2%) does not influence cell

viability. It reduces cell proliferation without coenzyme Q_{10} present to 23 ± 5%. A presence of 1 or 10 μM coenzyme Q_{10} in the medium with 2% serum stimulated cell proliferation by 16 ± 6%. No enhanced proliferation was observed with coenzyme Q_{10} at concentrations 0.1 or 100 μM. It is noteworthy that cell viability under serum deficiency increased with increased ubiquinone concentration. Longer cell cultivation (72 h) with 2% serum and 1 μM coenzyme Q_{10} is accompanied with increased proliferation rate (by 40 ± 7%). This shows that coenzyme Q_{10} increases the cell viability and proliferation with serum deficiency.

Figure 1 shows the distribution of JC-1 dye in C6 glioma cells exposed to 1 μM coenzyme Q_{10} for 20 min and 24 h. JC-1 (emission at 530 nm) is accumulated in energized mitochondria producing dimers (emission at 590 nm) (Smiley et al., 1991). The ratio of the fluorescence intensity of the dye in monomer and dimer forms (I_{590}/I_{530}) shows the mitochondrial membrane potential. Comparison of Figs. 1, I, e and 1, I, g shows that the mitochondrial potential is not altered after ubiquinone addition. The mitochondrial potential, as well as the number of functioning mitochondria, was reduced in cells cultivated for 24 h with serum deficiency (Figs. 1, I, (f), II). The presence of ubiquinone assisted in maintenance of the mitochondrial potential and increased the number of functioning mitochondria (Figs. 1, I, (f, h), II).

To clarify the role of redox processes in coenzyme Q_{10}-induced C6 cell proliferation under conditions of serum deficiency we studied the impact of ubiquinone

Table 1. Proliferation (*N*) and viability (*V*) of glioma cells exposed to coenzyme Q_{10} in various concentrations for 24 h

Coenzyme Q_{10}, μM	*N*		*V*	
	Serum content in culture medium,%			
	10	2	10	2
0	100 ± 5	77 ± 5	100 ± 4	96 ± 4
0.1	105 ± 5	79 ± 5	98 ± 3	99 ± 5
1	96 ± 6	93 ± 6[a]	102 ± 4	103 ± 4
10	100 ± 3	92 ± 5[a]	99 ± 3	113 ± 6[b]
100	99 ± 6	76 ± 5	90 ± 4	115 ± 7[b]

N and *V* are normalized for values in control cultures maintained under 10% serum in culture medium and absence of coenzyme Q_{10}. There are statistically significant changes relative to the samples without quinone treatment at [a] $p < 0.01$ and [b] $p < 0.05$.

on the intracellular content of GSH, a major low-molecular antioxidant. It was found that the level of GSH declined by 15 ± 5% in cells cultivated with 1 μM coenzyme Q_{10} for 24 h.

Quinone redox conversion in cells is mediated by enzymes catalyzing one- or two-electron reduction processes. One-electron reduction of quinone leading to semiquinone generates superoxide anion radical due to autoxidation of the latter. On the other hand, two-electron reduction of coenzyme Q_{10} to ubiquinol prevents ubisemiquinone production. It is known that ubiquinol displays antioxidant properties (Bentinger et al., 2007). Two-electron reduction of coenzyme Q_{10}

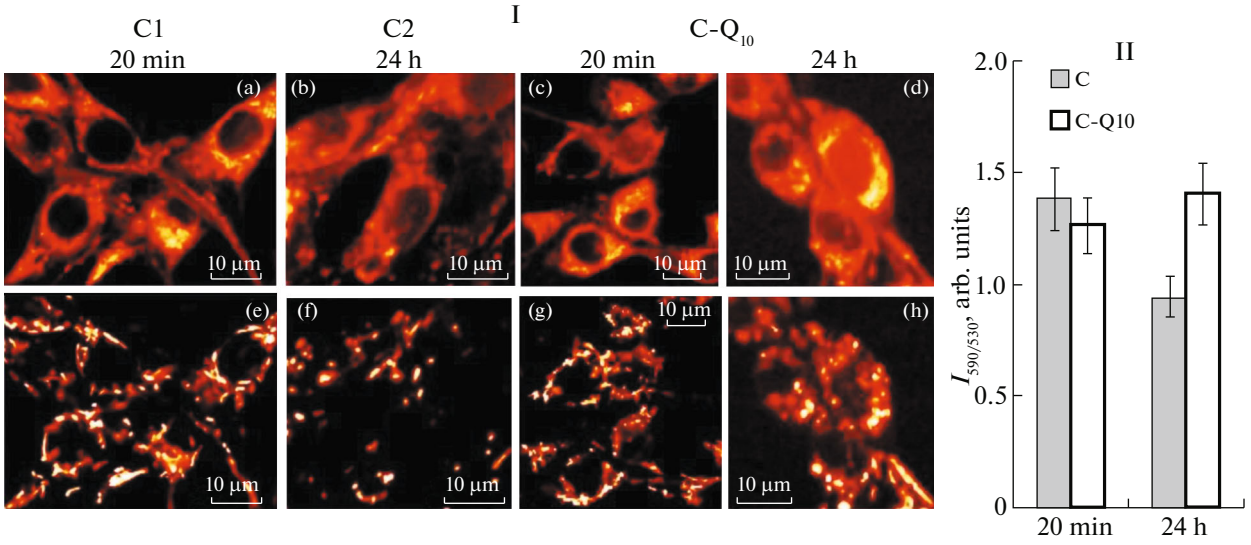

Fig. 1. Distribution of (I) JC-1 fluorescent dye and (II) mitochondrial potential (in C6 glioma cells exposed to 1 μM coenzyme Q_{10} (C-Q_{10}, 1 μM) under conditions of serum deficiency (2%). I—Confocal images: (a, b, c, d) fluorescence of monomer JC-1 (at 530 nm); (e, f, g, h) fluorescence of dimer JC-1 (at 590 nm); (a, c, e, g) standard culture conditions with absence of C-Q_{10} ((a, e) control C1) and presence of C-Q_{10} for 20 min (c, g); (b, d, f, h) cultivation for 24 h with serum deficiency and absence of C-Q_{10} ((b, f)—C2) and presence of C-Q_{10} (d, h). II—Mitochodrial potential is presented as the ratio of JC-1 fluorescence intensities at 590 and 530 nm (emission) in control (C) and with C-Q_{10} treatment for 20 min and 24 h under conditions of serum deficiency.

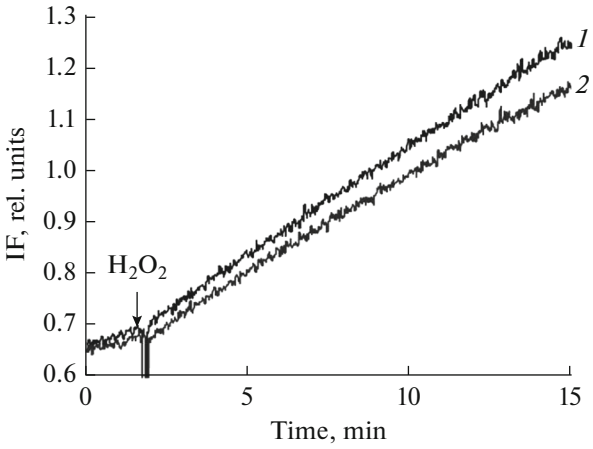

Fig. 2. Kinetic curves of fluorescence intensity (IF) of rat glioma cells loaded with H_2DCF-DA probe and exposed to 50 μM hydrogen peroxide with (curve *1*) absence or (curve *2*) presence (1 μM) of coenzyme Q_{10} in conditions of serum deficiency for 24 h.

in the plasma membrane is catalyzed with NADH-quinone oxidoreductase 1 (DT-diaphorase, NQO1) (Navarro et al., 1995; de Cabo et al., 2004), lipoamide dehydrogenase, thioredoxin reductase, and glutathione reductase. DT-diaphorase is regarded as a major enzyme (Nordman et al., 2003) catalyzing ubiquinone reduction to ubiquinol in the extramitochondrial fraction (Navarro et al., 1995; de Cabo et al., 2004).

We found that GSH concentration decreased by 22 ± 5% (relative to control) in cells cultivated for 24 h under inhibition of DT-diaphorase with dicoumarol. Supposedly, without two-electron reduction, coenzyme Q_{10} more effectively converts into ubisemiquinone. This results in increased ROS and a decreased GSH pool.

Reduced GSH concentration registered after coenzyme Q_{10} exogenous action may be mediated with a shifted chemical balance of one- and two-electron reduction, which results in semiquinone production. This is accompanied with further ROS generation and reduced GSH level in cells. On the other hand, coenzyme Q_{10} competes with GSH for binding sites in the active center of glutathione reductase, which also may diminish GSH content (Nordman et al., 2003).

The cellular redox state may be tested on the basis of the rate of scavenging of exogenous hydrogen peroxide (Makino, 1994; Krylova et al., 2008). Figure 2 shows typical kinetic curves of the fluorescence intensity in cells loaded with H_2DCF-DA (a probe for ROS production) and exposed to 50 μM H_2O_2. H_2O_2 penetrates into the cells, oxidizes the probe, and enhances the fluorescence intensity. On the other hand, H_2O_2 utilization with the cellular antioxidant system elicits a gradual decrease of the probe oxidation rate. This shows that increased antioxidant cell status will result in the reduced probe fluorescence observed with addition of exogenous H_2O_2. Figure 2 demonstrates that, in glioma cells cultivated with ubiquinone for 24 h and exposed to exogenous H_2O_2, the fluorescence intensity is lower than in control cells. This indicates a higher rate of H_2O_2 exogenous utilization after ubiquinone treatment. Thus, ubiquinone increases the intracellular antioxidant status of C6 cells maintained under serum deficiency.

We revealed that dicoumarol (NQO1 inhibitor) reduced the cell number by 35 ± 7% in cultures maintained under serum deficiency. Ubiquinone and dicoumarol together did not affect the glioma-cell proliferative activity (111 ± 11%, Table 2). This shows that the reduction of ubiquinone to ubiquinol is a prerequisite for the activation of cell proliferation.

The oxidation of quinol to semiquinone and quinone produces ROSs and activates redox-dependant factors involved in cell adaption to oxidative stress, which results in antioxidant accumulation. We used a JSH-specific inhibitor to analyze the implication of the nuclear factor Nf-κB in the regulation of glioma-cell proliferation. It was found that JSH reduced the cell number by 36 ± 5% with serum deficiency. Addition of coenzyme Q_{10} to the cells with inhibited Nf-κB increased the cell proliferation by 35 ± 9% comparative to cells cultivated without quinone. Thus, Nf-κB is not activated during ubiquinone-mediated cell proliferation under FBS deficiency.

Protein kinase C (PKC) and the mitogen-activated protein kinases ERK1/2, p38, JNK, and phosphotidylinositol-3-kinase (PI3K) are redox-regulated proteins involved in control of cell proliferation (Trachootham et al., 2008). In conditions of serum defi-

Table 2. Proliferative activity of glioma cells with signal-transduction inhibitors at treatment with 1 μM coenzyme Q_{10} for 72 h under conditions of serum deficiency

Target	Inhibitor	Proliferative activity, %		
		control	1 μM coenzyme Q_{10}	
		N_i/N_0	N_Q/N_0	N_Q/N_i
–	–	100 ± 5	140 ± 10	140 ± 10
NQO1	Dicoumarol	65 ± 7	72 ± 5	111 ± 11[b]
Nf-κB	JSH	64 ± 5	86 ± 5	135 ± 9
PKC	Gö6983	97 ± 7	93 ± 10	97 ± 11[a]
PI3K	LY294002	92 ± 10	90 ± 7	97 ± 11[a]
ERK1/2	PD098059	72 ± 9	70 ± 6	96 ± 12[a]
p38	Sb203580	67 ± 5	80 ± 6	120 ± 7[a]
JNK	SP600125	52 ± 5	68 ± 5	131 ± 10

N_0—cell proliferative activity in control with absence of both coenzyme Q_{10} and inhibitor; N_i—cell proliferative activity with presence of inhibitor, but absence of coenzyme Q_{10}; N_Q—proliferative activity of cells treated with coenzyme Q_{10} in the presence of inhibitors. [a] $p < 0.05$; [b] $p < 0.05$.

ciency, glioma-cell proliferation does not depend on PKC and PI3K, but it slows down with inhibited ERK1/2, p38, and JNK (Table 2). No ubiquinone-produced stimulated proliferation (96–97%, Table 2) was observed with the combined action of coenzyme Q_{10} and ERK1/2, PKC, or PI3K inhibitors. This shows that ERK1/2, PKC, and PI3K are involved in the transduction of the signal stimulating proliferation with participation of coenzyme Q_{10} in conditions of serum deficiency.

Coenzyme Q_{10} enhanced the cell proliferation with inhibited p38 and JNK. However, the relative increase in the cell number in control (with lack of inhibitors and coenzyme Q_{10}) was 40 ± 10%, whereas it was 20 ± 7% with inhibited p38 and 31 ± 10% with suppressed JNK (Table 2). This shows that the contribution of p38 and JNK kinases in coenzyme Q_{10}-mediated viability and/or proliferation is insignificant.

DISCUSSION

The mitochondrial potential of cells cultivated in a medium with serum deficiency declined, which is a common indicator of triggering of apoptosis or cell entry into the G_0 phase. We demonstrated that glioma-cell treatment with coenzyme Q_{10} restored the mitochondrial potential, increased the number of functioning mitochondria, and enhanced cell viability and proliferative activity. In other words, it prevents apoptosis and/or cell entry into the resting phase.

The high hydrophobicity of coenzyme Q_{10} impedes its intracellular distribution, and it accumulates mostly in and near the plasma membrane. Enzymes located in or near the plasma membrane are likely targets of the coenzyme Q_{10}. With nutrient deficiency, development of apoptosis is accompanied with ceramide accumulation and subsequent mitochondrial dysfunction (Ueda, 2015). Ubiquinone, which inhibits SMPD, prevents ceramide-induced apoptosis and stimulates cell growth under conditions of serum deficiency (López-Lluch et al., 1999; Gómez-Díaz et al., 2000; Navas et al., 2002). The mechanisms underlying the inhibiting effect of ubiquinone presumably are based on the direct interaction with the enzyme, as well as coenzyme-Q_{10}-induced alteration of the cellular redox state and inhibition lipid peroxidation (Martín et al., 2003; Navas et al., 2007). It has been reported that reduced coenzyme Q_{10} within the range of 10–100 μM is an effective noncompetitive inhibitor of SMPD in the plasma membrane, whereas in the oxidized form it inhibits SMPD much more weakly: with a dose of 10 μM, SMPD inhibition was no more than 20% (Martin et al., 2001). The enzyme activity reduced with increased concentration of ubiquinone. We revealed that the viability of cells cultivated with serum deficiency augmented monotonically with increasing concentration of coenzyme Q_{10}. Two-electron reduction of ubiquinone to ubiquinol is a prerequisite for the activation of cell proliferation. These findings suggest that ubiquinone-caused SMPD inhibition is a mechanism preventing apoptosis and inducing proliferation in glioma cells maintained in conditions of serum deficiency.

Unlike viability, coenzyme Q_{10}-induced proliferative activity of glioma cells altered within a limited range of concentrations (1–10 μM), rather than monotonically increasing with elevated quinone doses. This suggests that there exist additional mechanisms of ubiquinone-induced activation of cell growth.

Various signals of cell proliferation are redox-dependant. We found that cell treatment with coenzyme Q_{10} increased the antioxidant cell status (the rate of hydrogen-peroxide utilization increased), but, on the other hand, the pool of reduced glutathione diminished (cultivation for 24 h). The intracellular redox-system is based on the modification of sulfhydryl groups of redox-sensitive proteins; therefore, the reduced GSH pool that we detected may increase the sensitivity of redox-active players and their activation even with a low level of oxidants in spite of the elevated antioxidant cell status. It has been reported that glutathione is able to inhibit SMPD. Oxidized glutathione is a three times more effective SMPD inhibitor than reduced (Liu and Hannun, 1997). In addition, exogenous oxidized glutathione activates the epidermal growth-factor receptor (EGFR) (Burova et al., 2005).

Exposure of CaCo-2 cells to 50 μM coenzyme Q_{10} increased the expression of 79 genes encoding signaling proteins, including PKC. These findings confirm that coenzyme Q_{10} plays a regulatory role in cells (Groneberg et al., 2005). We found that PKCε, ERK1/2, and PI3K are involved in the stimulation of glioma-cell proliferation produced with coenzyme Q_{10}. PKC activation impedes ceramide production and ceramide-induced apoptosis (Mansat et al., 1997). PKC is able to activate sphingosine kinase (SphK) and translocate it from the cytosol to the membrane (Johnson et al., 2002). In turn, SphK activation stimulates sphingolipid metabolism with accumulation of sphingosine-1-phospate (S1P), enhancing cell proliferation via the EGF receptor triggering Ras/Raf/MEK/ERK1/2 (Kim et al., 2000) and PI3K/Akt (Wang, 2012) signaling. It should be noted that PKC-mediated activation of SphK/EGF/Ras/Raf/MEK/ERK1/2 and PI3K/Akt signaling is a mechanism of ubiquinone-stimulated glioma-cell growth under conditions of serum deficiency.

Stress-activated p38 and JNK kinases are activated predominately to trigger apoptosis (Davis, 2000; Wada and Penninger, 2004), in particular, that associated with ceramide accumulation (Chen et al., 2008). More and more studies show that these kinases are involved in maintenance of the viability and active proliferation of tumor cells and, therefore, in cancer development (Davis, 2000; Du et al., 2004; Thornton and Rincon,

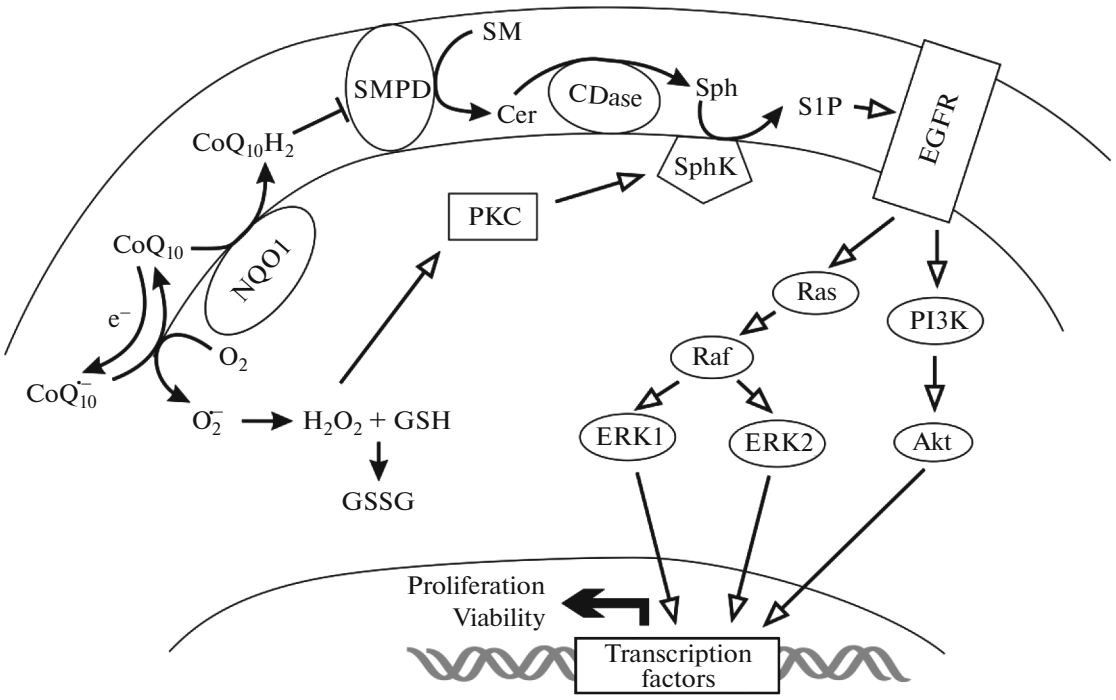

Fig. 3. Mechanisms underlying coenzyme Q_{10} regulation of glioma-cell viability under conditions of serum deficiency (2%). Coenzyme Q_{10} (CoQ10) is reduced with NAD(P)H:quinone oxidoreductase I (NQO1) to ubiquinol ($CoQ_{10}H_2$), which inhibits ceramide (Cer) accumulation and maintains cell viability under conditions of serum deficiency. Coenzyme Q_{10} shifts the chemical equilibrium of one- and two-electron reduction to semiquinone ($CoQ_{10}^{\cdot-}$) production. It is accompanied with additional H_2O_2 generation and decreased level of reduced glutathione (GSH) and protein kinase C (PKC) activation. PKC activates sphingosine kinase (SphK), resulting in sphingosine-1-phosphate (S1P) accumulation. S1P induces transactivation of epidermal growth-factor receptor (EGFR) and activation of Ras/Raf/MEK/ERK1/2 and PI3K/Akt. As a result, cell viability and proliferative activity are increased under conditions of serum deficiency. CDase—ceramidase, GSSG—oxidized glutathione, SM—sphingomyelin, Sph—sphingosine, PI3K—phosphoinositol-3-kinase.

2009). Thus, activation of JNK2 kinase accelerated KB-3 cell proliferation by reducing the time of cell transition from the S-phase of the cell cycle to G_2/M. The cell-growth inhibition that we have detected with suppressed p38 and JNK kinases show that these kinases facilitate tumor-cell viability during their long-term cultivation in a medium with serum deficiency.

Our findings and literature data suggest that the following mechanism of coenzyme Q_{10} action on cells maintained under nutrient deficiency exists (Fig. 3). Coenzyme Q_{10} that has reduced to ubiquinol inhibits SMPD and reduces the GSH pool, with subsequent activation of PKC and PKC-mediated activation of SphK. This results in the accumulation of sphingosin-1-phosphate (S1P) and prevention of ceramide production. A lack of ceramide provides cell viability and a high level of mitochondrial functional activity under serum deficiency. It hinders the triggering of apoptotic pathways. S1P induces EGFR transactivation with subsequent activation of the Ras/Raf/MEK/ERK1/2 and PI3K/Akt cascades, which enhances cell proliferation and viability in conditions of serum deficiency.

In conclusion, the results that we have obtained suggest that ubiquinone promotes glioma-cell viability and enhances the proliferation of tumor cells in areas with nutrient deficiency.

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