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EXPERIMENTAL PAPER

Expression of genes modulated by epigallocatechin-3-gallate in breast cancer cells

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Summary

Introduction: Breast cancer is the most common malignant cancer among women. Both drug resistance and metastasis are major problems in the treatment of breast cancer. Therefore, adjuvant therapy may improve patients' survival and affect their quality of life. It is suggested that epigallocatechin gallate (EGCG) which is well known for its chemopreventive activity and acts on numerous molecular targets may inhibit the growth and metastasis of some cancers. Hence, discovering the metastatic molecular mechanisms for breast cancer may be useful for therapy.

Objective: The aim of the study was to determine the effect of EGGC on the mRNA expression level of genes such as *ZEB1, ABCB1, MDM2, TWIST1* and *PTEN* in MCF-7 breast cancer cells.

Methods: MCF7/DOX were cultured in the presence of 0.2 μM DOX and EGCG (20-50 μM). The mRNA expression level was determined by real-time quantitative PCR using RealTime ready Custom Panel 96 kit.

Results: Our results showed an important increase (about 2-fold for 20 μM EGCG + 0.2 μM DOX and 2.5-fold for 50 μM EGCG + 0.2 μM DOX, $p<0.05$) in *ZEB1* expression levels. In case of *ABCB1* gene lack of influence on the mRNA level was observed ($p>0.05$). We also observed significant decrease of *ZEB1* expression in MCF7 cells with 20 μM and 50 μM EGCG ($p<0.05$). In addition, EGCG (20 μM) caused an increase of *MDM2* and *PTEN* mRNA levels in almost 100% ($p<0.05$) and 40% ($p>0.05$), respectively. Lack of the influence of EGCG was noted for the *TWIST1* gene expression. In case of MCF7/DOX we showed an increase of mRNA level of *PTEN* gene about 50% ($p<0.05$).

Conclusions: These results suggest that EGCG may be potentially used in adjuvant therapy in the breast cancer treatment.

Key words: ***breast cancer, EGCG, molecular study, expression, adjuvant therapy***

Słowa kluczowe: ***rak piersi, EGCG, badania molekularne, ekspresja, terapia adiuwantowa***

INTRODUCTION

Breast cancer is one of the most common cancers among women. The incidence reaches about 25% new cases per year [1, 2]. Although there is no effective drug for cancer, it is suggested that several ones can be prevented by healthy lifestyle, including diet modification. Many authors reported that some fruits and vegetables may negatively correlate with a risk of development of chronic diseases and several cancers [3-5]. Furthermore, the phytochemicals including phenolic compounds, carotenoids, contained in natural products have become a promising research topic in the prevention and treatment of cancer [6-8]. Recently, it was reported that the polyphenols are able to interrupt cellular signalling, especially NF-κB and AP1 pathways, by scavenging reactive oxygen species (ROS) responsible for their activation [9, 10].

(-)-Epigallocatechin-3-gallate (EGCG) is one of the most studied catechins, well known for its favorable biological properties, like antioxidative potential [11]. Moreover, EGCG may induce apoptosis and inhibit growth of many human cancers, for example leukemia, colon cancer and breast cancer [12, 13]. Additionally, the *in vivo* studies assessing the effectiveness of EGCG against cancers are not always comparable with *in vitro* models due to differences in bioavailability of EGCG and ability to bind with target in cell [14, 15]. Many studies showed EGCG may inhibit MCF-7 cells proliferation by influencing the transcript level of hypoxia-inducible factor 1α and vascular endothelial growth factor [16]. In addition, EGCG may exhibit its anti-proliferation activity by the modulation of the PI3K-Akt pathway and Bcl-2 proteins, inhibition of some kinases (Cdk2, Cdk4) and activation of Cdk inhibitors p21 and p27 [17, 18]. However, although there are some interesting data concerning the activity of polyphenolic compounds against tumour cells, up to now little is known about their properties for breast cancer cells towards evaluation of genes such as: zinc finger E-box binding homeobox 1 (*ZEB1*), ATP-binding cassette, sub-family B (*ABCB1*), MDM2 p53 binding protein homolog (*MDM2*), twist homolog 1 (*TWIST1*), phosphatase and tensin homolog (*PTEN*) involved in apoptosis and proliferation. Therefore, the aim of the study was to determine the influence of EGCG on the expression of *ZEB1, ABCB1, MDM2, TWIST1* and *PTEN* in MCF-7 breast cancer cells.

EXPERIMENTAL

Study design

Epigallocatechin gallate (EGCG) and doxorubicin (DOX) were provided by Sigma-Aldrich. The ER-positive breast cancer cell line, MCF-7 was obtained as a gift from Professor M. Zabel, Poznań University of Medical Sciences (Poland). The cell culture was carried out using Dulbecco's Modified Eagle Medium (DMEM-Gibco®) (Sigma-Aldrich), with 10% fetal bovine serum (Sigma-Aldrich) and 0.1% penicillin (100U/ml)/streptomycin (100 μg/ml) (Sigma-Aldrich) in incubator at 37°C with 5% CO_2 atmosphere.

MCF7/DOX were cultured in the presence of 0.2 μM DOX. All cultures (MCF7, MCF7/DOX, MCF7/EGCG and MCF7/DOX/EGCG) were initiated at a density of 4 × 100 cells/ml and grew for 160 h to reach the cell confluence. Thereafter, culture medium was removed and then cells were washed with PBS and incubated in culture medium without FBS in the presence of EGCG (20-50 μM) for 24 h, 48h, 72h, 96h, respectively. The effect of EGCG on cell growth and viability was assessed using a Bürker hemocytometer. Analysis of expression level of studied genes for different concentrations of EGCG was determined using real-time PCR.

Expression analysis

Total RNA extraction was performed using TriPure Isolation Reagent (Roche) according to the manufacturer's protocol. The quantitative assessment and purity of RNA were determined by a spectrophotometer (NanoDrop, Thermo Fisher Scientific). The cDNA synthesis was conducted from 2 μg of total RNA using Transcriptor First-Strand Synthesis System (Roche) according to the manufacturer's protocol. The gene expression *(ZEB1, ABCB1, MDM2, TWIST1, PTEN*) was determined using RealTime ready Custom Panel 96 kit (Roche). Real-time PCR was conducted with use of LightCycler® 96 Instrument (Roche, Germany) and a LightCycler® 480 Probes Master kit (Roche, Germany) according to the manufacturer's protocol. As a housekeeping gene we used the *ACTB* gene for quantitative expression. The data were assessed using LightCycler ® 96 software.

Statistical analysis

The obtained findings were expressed as mean ±SEM. Correlation analysis between the control and examined groups was performed using one-way ANOVA test. Value of $p<0.05$ was considered as statistically significant.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

In this work, we studied the influence of EGCG on the mRNA level of selected genes in MCF-7 cells after 96 h with 20 μM and 50 μM EGCG (fig. 1). By performing real-time PCR reaction, we have found that MCF7/DOX and MCF7/DOX/EGCG are characterized by important increase (about 2-fold for 20 μM EGCG + 0.2 μM DOX and 2.5-fold for 50 μM EGCG + 0.2 μM DOX, $p<0.05$) in *ZEB1* expression levels. In the case of *ABCB1* gene, we observed lack of influence on the mRNA level of this transporter as compared to control MCF-7 cells (fig. 1). What is interesting, we observed that the *ZEB1* expression significantly decreased in MCF7 cells with 20 μM and 50 μM EGCG ($p<0.05$). We also noted that EGCG (20 μM) caused an increase of *MDM2* and *PTEN* mRNA level about 100% ($p<0.05$) and 40% ($p>0.05$), respectively. Similar effect was noted for administration of 50 μM EGCG. In addition, we observed lack of the influence of EGCG on the *TWIST1* gene expression in MCF7 cells in comparison to control group. Furthermore, in case of MCF7/DOX we showed an increase of mRNA level of *PTEN* gene in about 50% ($p<0.05$). We also assessed the influence of EGCG on cell growth by the use of a Bürker hemocytometer. We showed that EGCG in the analyzed concentrations did not affect the cell growth of MCF7 without DOX within 3 days after EGCG administration (80-100% of control cell growth was observed in the presence of 20–50 μM EGCG) (fig. 2). However, we observed the inhibition of cell growth after 4 days in case of 20-50 μM EGCG administration.

DISCUSSION

Epidemiological studies show a relationship between green tea consumption and reduced risk of breast cancer [19, 20]. The widespread interest of EGCG

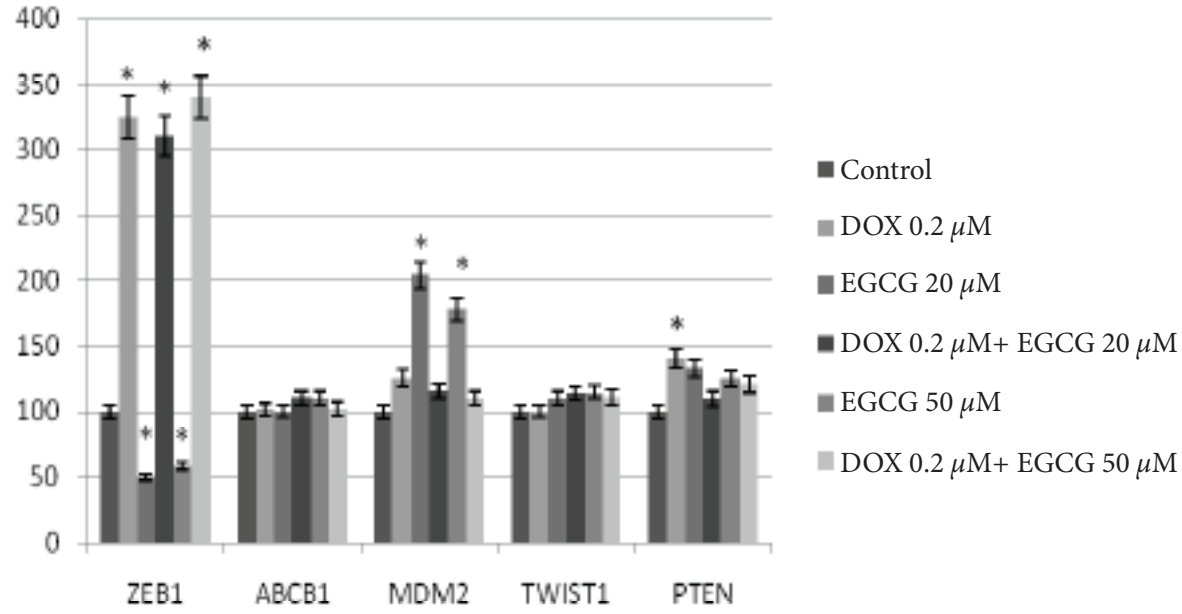

Figure 1.

Influence of epigallocatechin gallate (EGCG) and doxorubicin (DOX) on gene expression in MCF7 cells after 96 h. Control group was defined as 100%. Data were presented as mean ± SEM. *p<0.05 as compared with control group

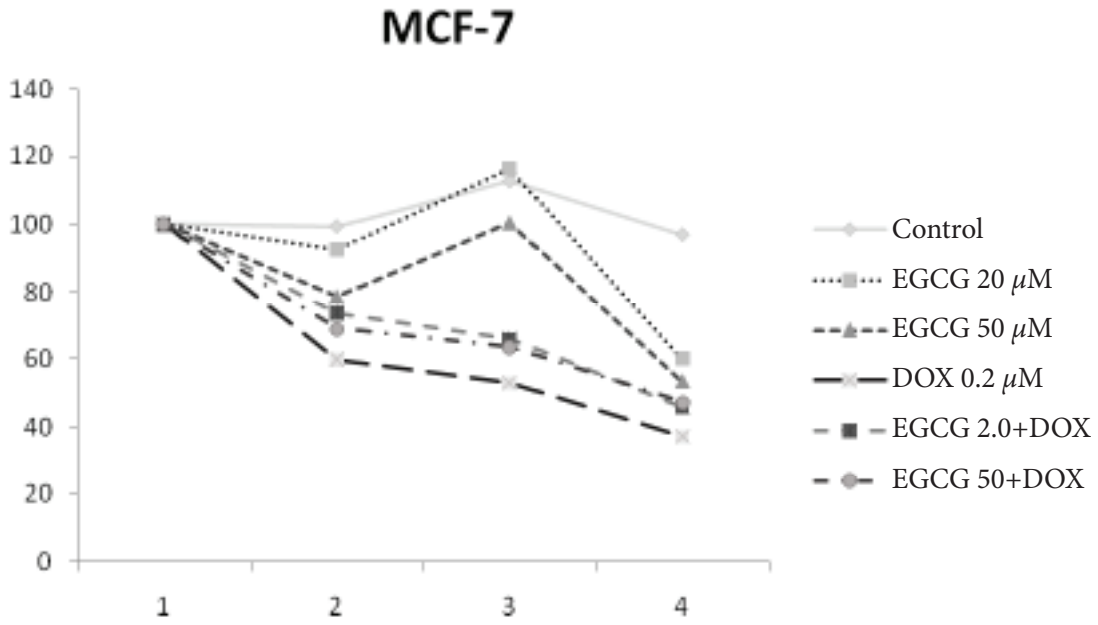

Figure 2.

Percentage of MCF7 cell survival after treatment with different concentrations of epigallocatechin gallate - EGCG (0, 20 and 50 μM), incubated for 4 days in presence or absence of doxorubicin-DOX (0.2 μM). Control group without EGCG and DOX

in prevention and treatment of breast cancer results from large number of data available based on *in vitro* and *in vivo* studies [21-23].

In our study, the effect of EGCG on the gene expression such as *ZEB1, ABCB1, MDM2, TWIST1* and *PTEN* in MCF-7 breast cancer cells was determined. We showed that MCF7/DOX and MCF7/DOX/EGCG are characterized by important increase in *ZEB1* expression levels in contrast to MCF7 cells with 20 μM or 50 μM EGCG alone. The *ZEB1* gene encodes a protein called zinc finger E-box-binding homeobox 1 involved in metastasis, treatment resistance and poor survival of patients with different cancers [24-26]. According to Brabletz *et al*., the increased *ZEB1* expression in breast cancer was observed both for the type of triple negative (ER-, PR-, HER2-) and basal types of this cancer [25, 27]. Hence, it is suggested that *ZEB1* gene and its expression can be a predictor for assessment of patient survival.

Analyzing *ABCB1* gene encoding P-glycoprotein (P-gp) responsible for the efflux of many anticancer drugs, which may lead to chemotherapy failure and multidrug resistance, we observed lack of EGCG influence on *ABCB1* expression in MCF-7 cells. Another study showed that EGCG reduced the expression level of *ABCB1* in a dose-dependent manner (20, 40 and 80 μg/ml) in the pancreatic PANC-1 cells, under hypoxic conditions [28]. However, these findings are ambiguous because hypoxia can also affect gene expression, including *ABCB1*.

We also determine the effect of EGCG on the mRNA expression of *MDM2* gene encoding a ring finger domain-containing protein. This protein may activation p53 ubiquitination as well as proteasomal degradation [29]. Moreover, the *MDM2* expression may be also modulate in a p53-dependent manner. It was shown that the increase of p53 strengthens the *MDM2* expression which induces degradation of p53. In our study we observed that EGCG leads to an increase of *MDM2* mRNA level. However, the mechanism of p53 regulation by EGCG requires further studies, because other researches showed an increase of p53 stability and a reduction of *MDM2* accumulation in the cell nucleus after EGCG treatment [30].

Furthermore, we also analyzed the expression level of *TWIST1* gene encoding the transcription factor *TWIST*, an oncoprotein which is an extensively studied regulator associated with cancer metastasis including breast cancer [31]. Another study showed that *TWIST1* is a direct target gene of miR-320 in the ovarian cancer cell. It is claimed that overexpression of miR-320 regulating gene expression by influence of translation and stability of target mRNAs inhibits the cell proliferation of ovarian cancer [32]. Our findings showed a lack of the impact of EGCG on the *TWIST1* gene expression in MCF7 cells. However, another study demonstrated that EGCG treatment reduces growth in human thyroid carcinoma cells depending on dose and causes a decrease of *TWIST* expression [33].

In addition, we determined the *PTEN* expression level as a potent suppressor gene involved in modulation of cellular functions, including proliferation, differentiation and migration. Activation of *PTEN* leads to inhibition of the PI3K/Akt/mTOR pathway responsible for tumour progression [34]. In our study, we showed that EGCG caused an increase of *PTEN* mRNA level in MCF7 cells in comparison to control group. It is suggested that EGCG may induce apoptosis in human breast cancer cells and influence their proliferation. Similar effect was obtained by Liu *et al.* indicating that EGCG leads to increase of *PTEN* expression and may inhibit the proliferation of pancreatic cancer cells. They also showed that EGCG may inhibit the PI3K/Akt/mTOR pathway by *PTEN* activation [34]. Another study also significantly showed an increase of the *PTEN* gene expression in variant T47D human breast cancer cells with high progesterone-receptor levels after EGCG treatment [35]. Hence, it is believed that EGCG may be potentially used in adjuvant therapy for the breast cancer treatment.

CONCLUSIONS

Our results provide new information on the effect of EGCG on the gene modulation associated with tumour cell proliferation, apoptosis and drug resistance. Additionally, based on our findings it may be suggested that a combination of EGCG with DOX treatment may enhance anticancer activity with no adverse side effects what may improve the quality of life in patients receiving chemotherapy. However, further studies are needed to provide evidence of practical application of EGCG in the cancers prevention and treatment.

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Conflict of interest: Authors declare no conflict of interest.

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