Effects of Somatostatin, Curcumin and Quercetin on the fatty acid profile of breast cancer cell membranes

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ABSTRACT

Breast cancer is a worldwide commonly found malignancy in women and effective treatment is regarded as a huge clinical challenge even in the presence of several treatment options. Extensive literature is available demonstrating polyphenols as phytopharmaceutical anticancer agents. Among the polyphenols; Quercetin and Curcumin have been reported to have a strong potential against breast cancer. However, so far, no comprehensive study has been performed to demonstrate the anticarcinogenic effects of Curcumin, Quercetin and their combinations with Somatostatin on the fatty acid profile of breast cancer cell membranes. We used MCF-7 and MDA-MB231 breast cancer cells incubated with Curcumin and Quercetin for 24h, in the absence and presence of Somatostatin, at their EC50 concentrations, to evaluate membrane fatty acid-based functional lipidomics together with the follow-up of EGFR and MAPK signaling pathways. The two cell lines gave different membrane free fatty acid reorganization: in MCF-7 cells, the following changes observed: increase of omega-6 linoleic acid in the cells incubated with Somatostatin+Quercetin and Quercetin and decrease of omega-3 acids in the cells incubated with Somatostatin+Curcumin compared to Somatostatin, and significant increases of monounsaturated fatty acid (MUFA), mono-trans arachidonic acid levels and docosapentaenoic acid for the cells incubated with Somatostatin+Quercetin compared to the control cells. In MDA-MB231 cells, incubations with Curcumin, Quercetin and Somatostatin+Quercetin induced the most significant membrane remodeling with the increase of stearic acid, diminution of omega-6 linoleic, arachidonic acids and omega-3 (docosapentaenoic and docosahexaenoic acids). Distinct signaling pathway changes were found for these cell lines. In MCF-7 cells, separate or combined incubations with Somatostatin and Quercetin, significantly decreased EGFR and incubation with Curcumin decreased MAPK signaling. In MDA-MB231 cells, incubation with Curcumin decreased AKT1 and p-AKT1(Thr308) levels. Incubation with Curcumin and Quercetin decreased the EGFR levels. Our results showed that cytostatic and antioxidant treatments can be combined to induce membrane fatty acid changes, including lipid isomerization as specific free radical-driven

process, and to influence signaling pathways. This study targeted to contribute to the literature on these antioxidants in the treatment of breast cancer, to clarify the effects and mechanisms in combination with Somatostatin.

Keywords: Somatostatin; curcumin; quercetin; breast cancer; cell signaling; membrane fatty acid profile

1. INTRODUCTION

Cancer is a deadly global disease and a critical barrier in cumulative life span of affected population (Bray et al. 2018). On the basis of recorded large death rate from breast cancer, it is estimated that more than 23 million people will suffer from this disease till 2030 (Bray et al. 2013). The majority of the breast cancer patients have a subtype that expresses estrogen receptors (ER) and progesterone receptors (PR) (Arena et al. 2019). There are different therapies available for the treatment of cancers, but many of them facing multiple problems of efficacy and safety which have already affected their overall therapeutic outcome and patient compliance (Park and Kim 2017). The international treatment guidelines and care are key to success in defecting breast cancer (Runowicz et al. 2016). Moreover, there is a need of new affordable and effective approaches to reduce death from breast cancer (Patel 2016). Natural compounds having high antitumor response, less toxicity and greater pharmacological or biological activities might provide therapeutic benefits in treating cancer (Watkins et al. 2015).

Among the large number of phytochemicals, polyphenols are the most studied showing great potential against cancer (Nabavi et al. 2018). Their apoptotic, autophagic, anti-inflammatory effects and maintaining redox balance make them promising anti breast cancer agents (Losada-Echeberria et al. 2017). Curcumin, genistein, quercetin, resveratrol and silibinin are reported to have great potential against breast cancer (Goncalves et al. 2017).

Curcumin is an active constituent of dried rhizomes of Turmeric (Curcuma longa) belonging to the ginger family. Curcumin from turmeric spice is a dietary polyphenol that exerts anticancer effects by acting on diversified molecular pathways involved in mutagenesis, apoptosis, tumorigenesis, cell cycle regulation, and metastasis. Curcumin has been found to block the spread of breast cancer cells to other parts of the body by blocking receptor activator of nuclear factor kappa-B ligand (RANKL) (Bharti et al. 2004) and also due to modulation of histone deacetylases (HDAC), histone acetyltransferases (HAT), DNMT1, and microRNAs in various cancer cells (Fu and Kurzrock 2010). Curcumin influences the sensitization of various chemotherapy drugs by regulating different signaling pathways, such as signal transducer and activator of transcription 3 (STAT3) (Fetoni et al. 2015), cell cycle modulators (Sivanantham et al. 2016) and BRCA (Chen et al. 2015). Quercetin, a

flavonol found in fruits and vegetables has been shown having antitumor activity through induction of P53, Caspase-3, -9, Cyt C in breast cancer cell lines. It was reported to enhance the activity of different drugs, including doxorubicin, cisplatin, and 5-fluorouracil (Daker et al. 2012; Samuel et al. 2012; Srinivasan et al. 2016). Researchers have explored combining curcumin with quercetin to improve their individual efficacy. Quercetin is known to block binding of albumin with curcumin and thus improves its bioavailability and potency.

Somatostatin produced by the delta cells of Langerhans islets, blocks the secretion of both insulin and glucagon (Harris 1994). In a recent study enhanced insulin and glucagon secretion was a result of decreased somatostatin secretion, showing that free fatty acids (FFAs) are regulators not only of insulin, but also of glucagon and somatostatin secretion (Kristinsson et al. 2017).

There is no information available in the literature demonstrating the potential effects of curcumin and quercetin on the fatty acid profile of breast cancer cell membranes.

In the light of multiple advantages of natural healing agents in this research article, we have focused on the effects of these agents in breast cancer, separately or in combination with somatostatin.

2. MATERIALS AND METHODS

2.1. Cell culture

Both cells were obtained from the ATCC. Curcumin and quercetin was purchased from Sigma. Somatostatin was purchased from Bachem. The cells were cultured in DMEM and RPMI supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin and 100 μg/ml of streptomycin in a 5% CO_2 atmosphere at 37°C. We determined the EC_{50} concentrations of somatostatin, curcumin and quercetin in MCF-7 and MDA-MB-231 cells. We incubated the MCF-7 cells with the EC_{50} concentrations of somatostatin (38.58 μM), curcumin (29.65 μM) and quercetin (73.63 μM) for 24 h. For MDA-MB-231 cells, EC_{50} concentrations of somatostatin was 96.75 μM, curcumin was 10.46 μM and quercetin was 92.98 μM. We applied these doses for the incubations of MDA-MB-231 cells. The standard protocols for cell culture studies were applied in all of the experiments and all the cell groups were prepared under the same conditions.

2.2. Phospholipid extraction and fatty acid analysis

Cells were detached using accutase, thoroughly washed with phosphate buffer, and after adding water pelleted by centrifugation at 14,000 ×g for 40 min at 4°C. Phospholipids were isolated from the pellet and fatty acids were derivatized to fatty acid methyl esters (FAME) as described which was followed by Gas Chromatographic (GC) analysis (Ferreri et al. 2005). Fatty acid compositions are

given in Table 1 and Table 2 as relative percentages of the total fatty acid content.

2.3. Mitogen-activated protein kinase (MAPK) assay

Cells were lysed with Cell Lysis Buffer (Cell Signaling Technology), then the levels of AKT1, p-AKT-1(Ser473), p-AKT-1 (Thr308), p-P44/42 MAPK (Thr202/Tyr204), S6-Ribozomal and p-S6-Ribozomal (Ser235/236) in the cell lysates were determined using the PathScan MAPK Multi-Target Sandwich ELISA Kit (Cell Signaling Technology) according to the manufacturer's instructions.

2.4. Epidermal growth factor receptor (EGFR) assay

Epidermal growth factor receptor (EGFR) levels were measured by sandwich ELISA kit (Biont), a sensitive colorimetric assay, in accordance with the procedure recommended by the manufacturer. The total protein content in the samples was determined by the Bradford method using bovine serum albumin (BSA) as a standard (Bradford 1976). The total protein content was used to normalize the EGFR and MAPK values of each sample.

2.5. Statistical analysis

Statistical analyses were conducted with SPSS software. Statistical significance was based on 95% confidence limits ($p \leq 0.05$). Comparison of the non-parametric data among the groups was performed using the Mann–Whitney U test. Results were given as mean±SD.

3. RESULTS

3.1. Membrane fatty acid profile

Table 1 shows membrane fatty acid composition in the MCF-7 cells after incubations with Somatostatin, Curcumin, Quercetin, Somatostatin+Curcumin and Somatostatin+Quercetin for 24h. Myristic acid (14:0) level, as a member of the saturated fatty acid (SFA) family, raised significantly in the Quercetin group compared to the control group, but diminished in the Curcumin and Somatostatin+Curcumin groups compared to the Somatostatin group. Somatostatin+Quercetin combination significantly diminished 14:0 level compared to the Quercetin group. Palmitic acid (16:0) decreased significantly in the Quercetin group (Table 1). MUFA, oleic acid (18:1, delta-9) decreased in the Somatostatin+Curcumin group compared to the control group and Somatostatin groups ($p<0.05$, $p<0.01$). Another MUFA 6cis-16:1 (sapienic acid) was found similar in all groups. 9cis-16:1 (palmitoleic acid) and 11cis-18:1 (cis-vaccenic acid) levels were significantly diminished in the Curcumin and Somatostatin+Curcumin groups, whereas they were significantly higher in the

Quercetin group in comparison to the control and Somatostatin groups. The level of the most important omega-6 essential fatty acid linoleic acid (18:2) significantly increased in the cells incubated with Quercetin and Somatostatin+Quercetin compared to the cells incubated with Somatostatin ($p<0.01$). On the other hand, in polyunsaturated fatty acid (PUFA) family, incubations with Somatostatin+Curcumin, Quercetin alone and Somatostatin+Quercetin combination significantly elevated 18:3 ω6 (γ- linolenic acid) and dihomo-γ-linolenic acid (DGLA, 20:3, ω6) levels compared to the control cells and Somatostatin cells. There was no change in the arachidonic acid level (20:4 ω6) in all cell groups. The marker of endogenous lipid isomerization, mono-trans arachidonic acid (20:4 t ω6) significantly elevated in the cells incubated with Somatostatin+Quercetin. The ω3 fatty acid docosapentaenoic acid (DPA, 22:5 ω3) level significantly increased in the cells incubated with Somatostatin, Quercetin alone or Somatostatin+Quercetin. It is worth underlining that a few changes were detected in MCF-7 cell membranes incubated with Curcumin and Somatostatin+Curcumin compared to the lipidome changes detected in the Quercetin and Somatostatin+Quercetin incubations.

Table 2 depicts membrane fatty acid composition in the MDA-MB231 cells incubated with Somatostatin, Curcumin, Quercetin, Somatostatin+Curcumin and Somatostatin+Quercetin for 24 h. The membrane fatty acid changes were not significant in the cells incubated with Somatostatin+Curcumin except for a long chain SFA 20:0 and 20:4t, whereas incubation with Curcumin caused significant increase of the ω3 DHA (22:6) and total SFA. There was no change in total trans fatty acid levels in all groups. Incubation with Somatostatin+Quercetin decreased the PUFA ω3 family involving DHA and was accompanied by the significant decrease in PUFA ω6 18:2. In contrast, SFA levels especially stearic acid significantly increased. The decrease of 20:4 ω6 fatty acid level in the MDA-MB 231 cells incubated with Curcumin was significant compared to the controls, and lower than the cells incubated with Somatostatin alone. In all treatments the 18:2 t isomer levels were similar. Fig.2. depicts the levels of SFA, MUFA, PUFA, total trans, total ω3 and total ω6 under experimental conditions. The membrane lipidome fatty acid monitoring showed that Curcumin, Quercetin and Somatostatin+Quercetin incubations caused an increase in the SFA levels compared to the control cells and cells incubated with Somatostatin.

Having these two cell lines in our hands, we could make also a comparative evaluation of the membrane lipidome changes, discovering that MDA-MB231 cells involved different fatty acids compared with MCF-7 cells, influencing MUFA and omega-6 in the latter whereas in the former SFA and omega-3 were mostly involved.

3.2. MAPK pathway member levels

Absorbances of AKT1, p-AKT-1(Ser473), p-AKT-1 (Thr308), p-P44/42 MAPK (Thr202/Tyr204), S6-Ribozomal and p-S6-Ribozomal (Ser235/236) in the MCF-7 cell lysates are shown in **Fig. 3**. MCF-7 cells were incubated with Somatostatin or Curcumin for 24h, leading to the inactivation of p-P44/42 MAPK (Thr202/Tyr204), p-S6-Ribozomal (Ser235/236) and AKT1 and activation of p-AKT1 (Thr308) and p-AKT-1 (Ser473). p-P44/42 MAPK (Thr202/Tyr204) pathway's level was significantly elevated in the Quercetin, Somatostatin+Curcumin and Somatostatin+Quercetin groups compared to the control group. The S6-Ribozomal was diminished by Somatostatin, whereas in the other treatments the S6-Ribozomal level showed an increase.

As shown in **Fig. 4**, 24h incubation of MDA-MB231 cells with Somatostatin, there was no significant difference in the p-S6-Ribozomal (Ser235/236) and p-AKT-1(Ser473) pathways in comparison to the control group. Somatostatin+Curcumin led to a decrease in p-S6-Ribozomal (Ser235/236) level compared to the Somatostatin and Curcumin groups. p-P44/42 MAPK (Thr202/Tyr204) level was significantly decreased in the breast cancer cells incubated with Somatostatin and Somatostatin+Curcumin. AKT1 level was increased significantly by SOMATOSTATIN treatment, whereas the Curcumin, Quercetin, Somatostatin+ Curcumin and Somatostatin+Quercetin combination decreased this level.

3.3. EGFR levels

As shown in **Fig. 5**, 24h incubation of MCF-7 cells with Somatostatin diminished the level of EGFR in the breast cancer cells compared to the control cells, instead Curcumin led to an increase in EGFR levels. **Fig. 6** depicts the level of EGFR in MDA-MB231 cell. The EGFR level was not significantly changed compared to controls except in the Curcumin, Quercetin and Somatostatin+Quercetin cells. EGFR levels were significantly decreased in the Curcumin and Quercetin groups compared to the controls.

4. DISCUSSION

We used two breast cancer cell lines, MCF-7 and MDA-MB231, in order to investigate the membrane fatty acid changes and signaling pathways related to Somatostatin, Curcumin and Quercetin, treatments, alone or in combination. The results of the membrane lipidome analysis are related to the well-known role of fatty acids and phospholipid formation for cancer growth, starting from the simple evidence that cells cannot live without membranes. The need for fatty acids is represented by an increased de novo lipogenesis with formation of palmitic acid and subsequent

elongation/desaturation steps, forming all the other components of the SFA and MUFA families (Igal 2011; Kamphorst et al. 2013).

In the membrane lipidome, the detection of trans fatty acids can be informative of free radical stress, that occurs also during chemotherapies, and the role played by antioxidant treatments, as shown previously by us in case of Ntera-2 human testicular germ cancer cells treated with bleomycin and several antioxidants (Cort et al. 2016). Our results reported in Table 1 and Table 2 showed an initial difference between MCF-7 and MDA-MB231 cell lines that was not known previously. In the MCF-7 cells the relative percentages of SFA found in the membrane phospholipids are similar, whereas the MUFA/PUFA ratio is different. The higher percentage of PUFA in MDA-MB231 cells indicates a different membrane arrangement. This is an important difference since the PUFA moieties of phospholipids are involved in the signaling and their role for cancer risk and progression is highly debated. The higher percentage of arachidonic acid in the MDA-MB231 cells than in the MCF-7 cells predisposes the cell signaling toward more inflammatory outcomes. Then, it is important to see that the effects of all treatments on the PUFA moieties resulted to be different either for the cell type and for the fatty acids involved: 1) as reported in Table 1 and Fig 1, the levels of total PUFA ω6 and ω3 in MCF-7 cells under our experimental conditions increased significantly in the combined Somatostatin+Curcumin incubation compared to the Somatostatin cells. The ω3 PUFA increase was seen also in the Somatostatin group compared to control group, whereas Curcumin alone did not cause any change in the membrane fatty acid families. This is an important element that suggests to deepen the Curcumin interaction with membrane lipids; 2) as shown in Table 2 and Fig 2, in MDA-MB231 cells treated with Curcumin, the effect on ω3 PUFA was the opposite of MCF-7 cells, since this family diminished, involving DPA and DHA, and the ω6/ω3 ratio changed in favor of the ω6 PUFA. Such changes were not observed in the separated other experiments. This result suggests that, depending on the cell type, the Somatostatin or Curcumin interaction can give different membrane reorganization and this can greatly influence biological and pharmacological outcomes; 3) beside the PUFA moieties, the other families involved in the lipidome changes were not the same for MCF-7 and MDA-MD231 cells: in the former the MUFA family significantly decreased, in the latter the significant increase of the SFA family levels were observed (Table 1 and Table 2).

Curcumin is one of the most extensively studied phytochemical for various biological activities due to its antioxidant (Abdel-Daim and Abdou 2015) and anticancer (Jin et al. 2015) properties. Curcumin is also shown having anticancer activities in a wide range of cancers, including pancreatic (Epelbaum et al. 2010), colon (Chauhan 2002), breast (Khazaei Koohpar et al. 2015), prostate (Chen 2015), and bladder (Liu et al. 2011) cancers. Curcumin is known to target multiple signaling

pathways and multiple genes that are involved in cancer growth, survival, and metastasis. Nagaraju and colleagues have recently summarized the molecular pathways targeted by curcumin (Nagaraju et al. 2012). Curcumin is known to target cyclin D1, c-myc (proliferation pathway), JNK, Akt, and AMPK (protein kinase pathway), Bcl-2, Bcl-xL, cFLIP, XIAP, and c-IAP1 (cell survival pathway), p53 and p21 (tumor suppressor pathway), caspase-8, −3, and −9 (caspase activation pathway), and DR-4, and −5 (death receptor pathway). Curcumin also found to inhibit epidermal growth factor receptors. Apart from these beneficial properties, Curcumin has not yet become an anticancer drug because of its high lipophilicity, low water solubility and metabolic instability (Padhye et al. 2010). Several drug delivery systems, as well as combination therapies, are designed to overcome these problems which achieved limited success (Hani and Shivakumar 2014). Numerous chemical derivatives of curcumin have been synthesized to overcome the problems of water solubility, metabolic instability and to enhance the potency of curcumin. Curcumin showed promising results in preclinical models, while its clinical efficacy is still under investigation. The results obtained following EGFR and MAPK pathways are shown in Fig 3, Fig 4, Fig 5, Fig 6 and the cascades of signals do not behave always similarly. Somatostatin is known to interact with its receptors in MCF-7 and MDA-MB231 cells inducing apoptosis with the status of MAPK signaling modulated in a cell-specific manner. As far as the EGFR level is concerned (Fig 5), Somatostatin was the only treatment efficient to reduce the EGFR level in the MCF-7 cells, whereas Curcumin increased it in the MCF-7 cells. In our experiments, the survival MAPK pathways were diminished in MDA-MB231 breast cancer cell lines incubated with Somatostatin, and Somatostatin+Curcumin, but MAPK signaling increased in MDA-MB231 cells incubated with Curcumin, Quercetin and Somatostatin+Quercetin. The rest of the signaling pathway was affected distinctively by the conditions used: p-AKT1 (Thr308) level significantly increased in MCF-7 cells incubated with all treatments, whereas the p-AKT1 (Ser473) seemed to be more sensitive (increased) to the treatments in the MDA-MB231 cells. The phosphorylation of AKT is an important link in the cell apoptosis process**.** Phosphorylated AKT (p-AKT) proteins cause cell death and regulate various signaling pathways (Gu et al. 2016). It is worth underlining here that in our study increased cancer cell proliferation due to incubation with all treatments was evidenced by MTT viability assay**.**

In this study, we investigated the effects of curcumin, quercetin alone or in combination with somatostatin on the fatty acid profile of breast cancer cell membranes**.** We explored the efficacy of quercetin and curcumin individually and in combination with somatostatin on the fatty acid profile in breast cancer cell lines**.** Having shown that in our experiments both level of activation of proliferative cascades and fatty acid quality display specific modulation by each treatment, we can

suggest the interplay between membrane composition and signaling as a crucial signature for the cell fate.

5. CONCLUSION

MCF-7 and MDA-MB231 breast cancer cells incubated with Curcumin and Quercetin for 24h, in the absence and presence of Somatostatin, at their EC50 concentrations, gave different membrane free fatty acid reorganization: in MCF-7 cells, the following changes observed: increase of omega-6 linoleic acid in the cells incubated with Somatostatin+Quercetin and Quercetin and decrease of omega-3 acids in the cells incubated with Somatostatin+Curcumin compared to Somatostatin, and significant increases of monounsaturated fatty acid (MUFA), mono-trans arachidonic acid levels and docosapentaenoic acid for the cells incubated with Somatostatin+Quercetin compared to the control cells. In MDA-MB231 cells, incubations with Curcumin, Quercetin and Somatostatin+Quercetin induced the most significant membrane remodeling with the increase of stearic acid, diminution of omega-6 linoleic, arachidonic acids and omega-3 (docosapentaenoic and docosahexaenoic acids). In MCF-7 cells, separate or combined incubations with Somatostatin and Quercetin, significantly decreased EGFR and incubation with Curcumin decreased MAPK signaling. In MDA-MB231 cells, incubation with Curcumin decreased AKT1 and p-AKT1(Thr308) levels. Incubation with Curcumin and Quercetin decreased the EGFR levels.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the study.

AUTHOR CONTRIBUTIONS

C.F. and T.O. formulated the idea and prepared the manuscript; C.C., C.F., T.O., S.S. designed the methodology and the research plan; A.H., F.H., E. K., G.M. performed experiments and organized the data and prepared all graphics; E.K., A.S., T.O., C. F., S.S., GDB supervised the data acquisition; G.D.B., S.S., C.C., T.O., C.F. discussed the results. All authors participated in the data discussion and ameliorated the draft. C.F. and T. O. acquired financial support.

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Table Legends

Table 1: Fatty acid methyl esters (FAME) obtained from membrane phospholipids of MCF-7 cells incubated with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h and from cells in the same conditions but not treated with these agents. The values are reported as the relative percentage (% rel.) of the total fatty acid peak areas detected in the GC analysis. The values are given as mean ± SD.

Table 2: Fatty acid methyl esters (FAME) obtained from membrane phospholipids of MDA-MB231cells incubated with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h and from cells in the same conditions but not treated with these agents. The values are reported as the relative percentage (% rel.) of the total fatty acid peak areas detected in the GC analysis. The values are given as mean ± SD.

Figure Legends

Figure 1: MUFA, PUFA, SFA, total ω3, total ω6 and total trans fatty acid moieties in the MCF-7 Cell Membranes Following Incubations with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h (n=6 in each experiment). Values are mean±SD. See Table 1 for values and complete analysis. *,**Values significantly different from the control $p < 0.05$, $p < 0.01$. $^{\&,\&\&}$ Values significantly different from the Quercetin $p<0.05$, $p < 0.01$. $^{\#,\#\#}$ Values significantly different from the Somatostatin $p < 0.05$, $p < 0.01$. $^{+,+\ +}$Values significantly different from the Curcumin $p < 0.05$, $p < 0.01$

Figure 2: MUFA, PUFA, SFA, total ω3, total ω6 and total trans fatty acid moieties in the MDA-MB231 cell membranes following incubations with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h (n=6 in each experiment). Values are mean±SD. See Table 2 for values and complete analysis.*,**Values significantly different from the control $p < 0.05$, $p < 0.01$. $^{\&,\&\&}$ Values significantly different from the Quercetin $p<0.05$, $p < 0.01$. $^{\#,\#\#}$ Values significantly different from the Somatostatin $p < 0.05$, $p < 0.01$. $^{+,+\ +}$Values significantly different from the Curcumin $p < 0.05$, $p < 0.01$

Figure 3: Absorbances of the MAPK pathway members; AKT1, p-AKT-1(Ser473), p-AKT-1 (Thr308), p-P44/42 MAPK (Thr202/Tyr204), S6-Ribozomal and p-S6-Ribozomal (Ser235/236) measured in the MCF-7 cells after incubations Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24 h (n=6.*,**Values significantly different from the

control p <0.05, p < 0.01. [&,&&] Values significantly different from the Quercetin p<0.05, p <0.01. [#,##] Values significantly different from the Somatostatin p <0.05, p <0.01. [+,+ +]Values significantly different from the Curcumin p <0.05, p <0.01

Figure 4: Absorbances of the MAPK pathway members; AKT1, p-AKT-1(Ser473), p-AKT-1 (Thr308), p-P44/42 MAPK (Thr202/Tyr204), S6-Ribozomal and p-S6-Ribozomal (Ser235/236) measured in the MDA-MB231 cells after incubations Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24 h (n=6). .[*,**]Values significantly different from the control p <0.05, p < 0.01. [&,&&] Values significantly different from the Quercetin p<0.05, p <0.01. [#,##] Values significantly different from the Somatostatin p <0.05, p <0.01. [+,+ +]Values significantly different from the Curcumin p <0.05, p <0.01

Figure 5: EGFR levels in MCF-7 cells after incubation for 24h with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin (n=6). .[*,**]Values significantly different from the control p <0.05, p < 0.01. [&,&&] Values significantly different from the Quercetin p<0.05, p <0.01. [#,##] Values significantly different from the Somatostatin p <0.05, p <0.01. [+,+ +]Values significantly different from the Curcumin p <0.05, p <0.01

Figure 6: EGFR levels in MDA-MB231 cells after incubation for 24h with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin (n=6). [**]Values significantly different from the control p <0.01. .[*,**]Values significantly different from the control p <0.05, p < 0.01. [&,&&] Values significantly different from the Quercetin p<0.05, p <0.01. [#,##] Values significantly different from the Somatostatin p <0.05, p <0.01. [+,+ +]Values significantly different from the Curcumin p <0.05, p <0.01

FAME[a]	Control	Somatostatin	Curcumin	SST+Cur	Quercetin	SST+Que
14:00	1.92±0.73	2.51±0.24	1.49±0.89	1.62±0.60 #	2.84 ± 0.37 *	1.98 ± 0.78 &
16:00	26.23±4.26	23.35±2.37	24.53±9.79	24.13±2.41	20.58 ± 1.06 **	22.27 ± 4.36
16:1 6 c	1.25±0.45	1.52±0.02	1.39±0.92 #	1.82±0.82	1.78 ± 0.25	1.65 ± 0.29
16:1 9 c	3.81±1.18	4.76±0.32	2.52±1.51	2.54±0.85 #	6.73 ± 0.58 **, ##	5.78 ± 0.78 *,&
18:00	10.40±2.50	11.93±0.43	13.65±6.20	14.79±4.12 *	11.52 ± 1.08	12.33 ± 0.66 *
18:1 9 trans	0.04±0.09	tr	0.35±0.71	0.17±0.09 *, #	tr ###	0.15 ± 0.26
18:1 9 c	27.89±2.96	26.89±1.21	27.47±2.94	23.49±2.45 **, #,+	25.48 ± 1.13	25.75 ± 0.88*
18:1 11 c	6.49±1.98	7.84±0.58	5.73±2.17 #	5.54±0.85 #	8.34 ± 0.67 *	7.79 ± 0.83
18:2 trans	0.30±0.14	0.31±0.14	0.63±0.46	0.20±0.22	tr **, ##	0.28 ± 0.08 &&
18:2 ω6	5.70±3.39	3.44±0.06	3.87±1.16	3.95±0.57	5.44 ± 2.60 ##	4.50 ± 0.27 ##
18:3 ω6	0.11±0.05	0.11±0.06	0.19±0.11	0.93±1.31 *	0,41 ± 0,10 **, ##	0.34 ± 0.06 **, ##
18:3 ω3	0.30±0.09	0.37±0.05	0.33±0.13	0.21±0.05 #	0.38 ± 0.37	0.27 ± 0.10
20:00	0.41±0.22	0.37±0.14	0.53±0.46	1.15±1.05	0,49 ± 0,14	0.46 ± 0.10
20:1 11c	0.91±0.35	0.95±0.07	1.12±0.65	1.00±0.64	1,35 ± 0,09 #	1.39 ± 0.24 *, ##
20:2w6	1.01±0.38	0.98±0.40	1.35±0.53	1.88±2.51	0,50 ± 0,05 *, ##	0.52 ± 0.08 *, ##
DGLA w6	0.97±0.26	1.0±0.05	2.68±2.51	1.62±0.14 **, #	1,40 ± 0,23 *, #	1.50 ± 0.22 *, #
20:4 trans ω6	0.03±0.02	0.02±0.03	0.06±0.11	0.51±0.99	tr **	0.25 ± 0.03**, #,&&
20:4 ω6	5.48±1.01	5.96±0.28	4.37±3.20	4.41±2.20	6.16 ± 0.42	6.14 ± 0.86
EPA	1.22±0.37	1.52±0.08	1.22±0.71	1.92±1.05	1.16 ± 0.14 ##	1.22 ± 0.14 ##
22:00	0.74±0.87	0.69±0.50	0.81±1.26	0.61±0.15	0.51 ± 0.14	0.61 ± 0.16
DPA	1.65±0.50	2.15±0.08 **	2.42±1.05	2.87±1.89	2.09 ± 0.17 **	2.19 ± 0.41 *
DHA	2.47±0.51	2.81±0.08	2.49±0.60	3.48±1.65	2.84 ± 0.19	2.63 ± 0.41
tot SFA	39.70±2.55	38.85±3.10	41.02±6.00	42.29±2.69	35.94 ± 0.93	37.65 ± 3.84
tot MUFA	40.53±1.00	42.13±2.11	38.77±1.84 #	34.83±4.29 **, #	43.67 ± 2.25 *	42.51 ± 1.67 *
tot PUFA	19.77±3.18	18.93±1.12	19.61±4.60	22.00±2.76	20.38 ± 2.52	19.84 ± 2.31
Tot ω6	13.60±3.66	11.81±0.85	13.15±2.54	13.51±1.73	13.69 ± 1.80 #	13.53 ±1.40 #
Tot ω3	5.89±1.59	6.84±0.18 **	6.46±2.20	8.48±1.74 **, #	6.48 ± 0.29 #	6.30 ± 0.92
tot trans	0,36± 0,25	0.32±0.17	1.04±0.76 *,#	0,88±0,97 #	tr **, ##	0.68 ± 0.30 *, #,&&

Table 1: Fatty acid methyl esters (FAME) obtained from membrane phospholipids of MCF-7 cells incubated with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h and from cells in the same conditions but not treated with these agents. The values are reported as the relative percentage (% rel.) of the total fatty acid peak areas detected in the GC analysis. The values are given as mean ± SD.

FAME[a]	Control	Somatostatin	Curcumin	SST+Cur	Quercetin	SST+Que
14:00	1.18 ± 0.43	1.23 ± 0.12	1.97 ± 0.16 **, ##	1.72 ± 0.13 **, ##,+	1.38 ± 0.18	1.60 ± 0.23 #
16:00	17.93 ± 5.46	18.84 ± 3.09	25.9 ± 1.24 **, ##	25.30 ± 2.99 **, ##	25.75 ± 2.19 **, ##	30.95 ± 2.88**, ##,&
16:1 6 c	1.93 ± 0.50	2.03 ± 0.38	1.81 ± 0.16	1.96 ± 0.28	1.94 ± 0.22	1.54 ± 0.13 #,&&
16:1 9 c	1.74 ± 0.52	1.75 ± 0.39	1.58 ± 0.16	1.49 ± 0.22	1.32 ± 0.33 #	1.05 ± 0.25 **, ##
18:00	18.99 ± 1.31	19.04 ± 1.40	21.69 ± 1.39 *, #	21.53 ± 2.91	21.93 ± 2.44 *, #	24.61 ± 2.06 **, ##
18:1 9 trans	0.09 ± 0.10	0.14 ± 0.14	tr #	tr #,+++	0.04 ± 0.09	tr #
18:1 9 c	22.27 ± 0.96	22.26 ± 0.56	17.62 ± 0.91 **, ##	16.48 ± 1.69 **, ##	14.24 ± 2.03 **, ##	11.56±0.91 **, ##,&&
18:1 11 c	6.28 ± 0.24	6.42 ± 0.08	5.12 ± 0.16 **, ##	5.3 ± 0.64 **, ##	4.64 ± 0.52 **, ##	3.61 ± 0.20 **, ##,&&
18:2 trans	0.24 ± 0.21	0.35±0.20	0.45 ± 0.05	0.38 ± 0.32	0.45 ± 0.17	0.56±0.10
18:2 ω6	4.75 ± 0.87	4.61 ± 0.36	4.55 ± 0.26	4.98 ± 0.53	4.51 ± 0.41	3.74 ± 0.38 *, ##,&
18:3 ω6	0.30 ± 0.13	0.26 ± 0.10	0.32 ± 0.02	0.52 ± 0.29 #	0.32 ± 0.05	0.27±0.05
18:3 ω3	0.37 ± 0.15	0.31 ± 0.10	0,25 ± 0,05	0.47 ± 0.20 +	0.29 ± 0.05	0.48 ± 0.60
20:00	0.62 ± 0.17	0.41 ± 0.22 *	0.36 ± 0.12 *	0.49 ± 0.23	0.53 ± 0.11	0.71 ± 0.56
20:1 11c	0.59 ± 0.15	0.54 ± 0.12	0.42 ± 0.10 *	0.52 ± 0.15	0.50 ± 0.06	0.67 ± 0.64
20:2w6	0.73 ± 0.18	0.64 ± 0.24	0.77 ± 0.10	0.90 ± 0.15 #	0.82 ± 0.21	0.64±0.20
DGLA w6	1.92 ± 0.47	1.88 ± 0.22	1.84 ± 0.14	1.82 ± 0.26	2.02 ± 0.25	1.82 ± 0.25
20:4 trans ω6	0.08 ± 0.13	0.16±0.18	0.28 ± 0.08 *	0,15 ± 0,13	0.29 ± 0.04 **	tr &&
20:4 ω6	8.50 ± 1.68	8.40 ± 0.96	6.66 ± 0.39 *, #	7.08 ± 0.98 #	8.87 ± 1.18	7.17 ± 1.02 &
EPA	1.80 ± 0.62	1.75 ± 0.44	1.35 ± 0.17	1.17 ± 0.20	1.14 ± 0.12 *, #	1.16 ± 0.45 #
22:00	0.58 ± 0.49	0.53 ± 0.12	0.23 ± 0.04 # #	0.19 ± 0.17 ##	0.42 ± 0.18	0.62 ± 0.76
DPA	4.29 ± 0.88	3.92±0.80	3.12 ± 0.17 *	3.72 ± 0.64	4.55 ± 0.81	4.00 ± 0.66
DHA	4.51±0.72	4.16 ± 0.66	3.69 ± 0.39 *	3.85 ± 0.73	4.06 ± 0.68	3.49 ± 0.41*, #
tot SFA	39.29 ± 5.42	40.06 ± 2.11	50.16 ± 1.80 **, ##	49.22 ± 5.84 **, ##	50.01 ± 3.31 **, ##	58.50 ± 3.67 **, ##,&&
tot MUFA	32.91±0.99	33.14±0.73	26.55 ± 1.23 **, ##	25.75 ± 2.69 **, ##	22.67 ± 2.87 **, ##	18.09 ± 1.18 **, ##,&&
tot PUFA	27.55±4.82	26.44±2.67	23.29 ± 1.12	25.03 ± 3.20	27.32 ± 3.32	23.09 ± 2.78 *, #,&
Tot ω6	16.54±2.90	16.29±1.59	14.88±0.58	14.29± 1.70	17.28 ± 1.82	13.73±1.41 #,&
Tot ω3	10.97±2.14	10.13±1.56	8.41 ± 0.67 *	9.21± 1.45	10.05 ± 1.55	9.13±1.57
tot trans	0,42±0,36	0,64±0,39	0.74 ± 0.12	0.53 ± 0.44	0.79 ± 0.24	0.47 ± 0.25

Table 2: Fatty acid methyl esters (FAME) obtained from membrane phospholipids of MDA-MB231cells incubated with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h and from cells in the same conditions but not treated with these agents. The values are reported as the relative percentage (% rel.) of the total fatty acid peak areas detected in the GC analysis. The values are given as mean ± SD.

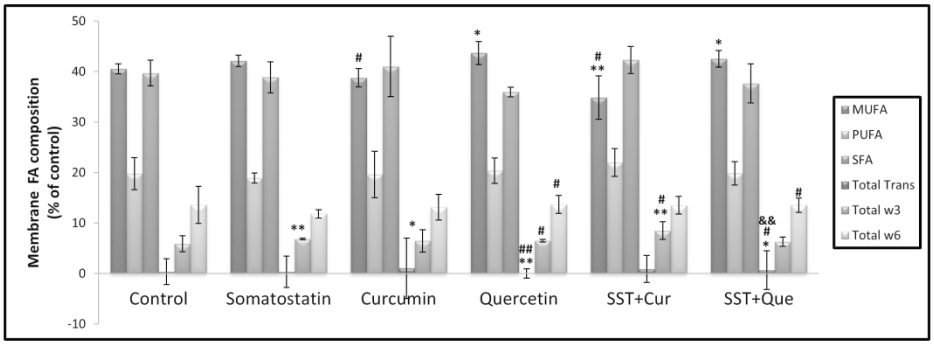

Figure 1: MUFA, PUFA, SFA, total ω3, total ω6 and total trans fatty acid moieties in the MCF-7 Cell Membranes Following Incubations with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24h (n=6 in each experiment). Values are mean±SD. See Table 1 for values and complete analysis. *,**Values significantly different from the control p <0.05, p < 0.01. &, && Values significantly different from the Quercetin p<0.05, p <0.01. #,## Values significantly different from the Somatostatin p <0.05, p <0.01. +,+ +Values significantly different from the Curcumin p <0.05, p <0.01

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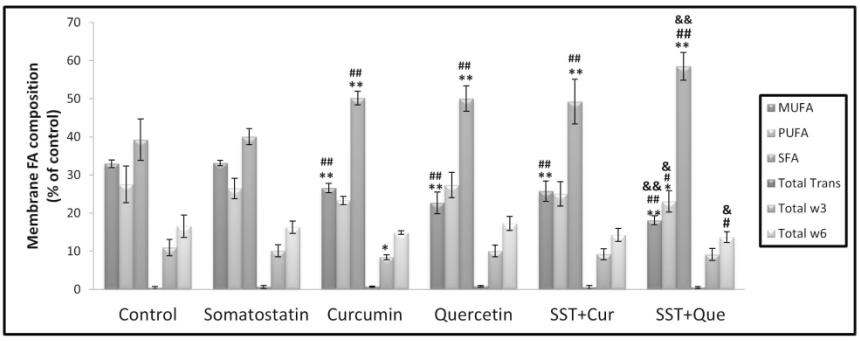

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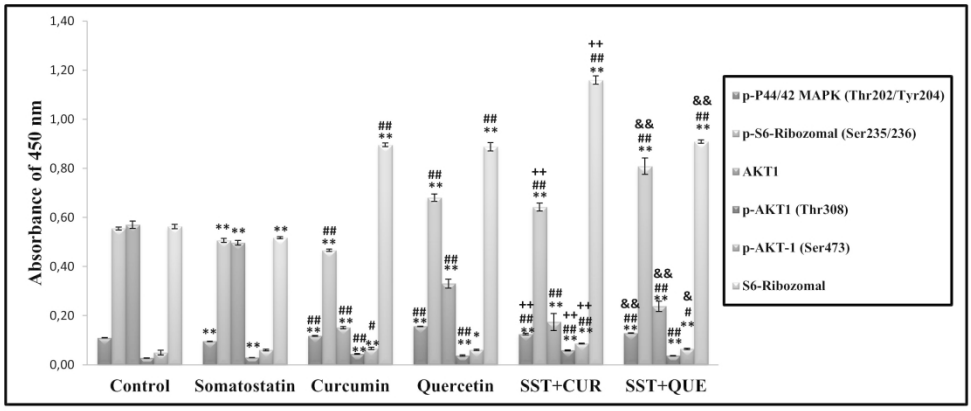

Figure 3: Absorbances of the MAPK pathway members; AKT1, p-AKT-1(Ser473), p-AKT-1 (Thr308), p-P44/42 MAPK (Thr202/Tyr204), S6-Ribozomal and p-S6-Ribozomal (Ser235/236) measured in the MCF-7 cells after incubations Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin for 24 h (n=6.*,**Values significantly different from the control $p < 0.05$, $p < 0.01$. &,&& Values significantly different from the Quercetin $p<0.05$, $p < 0.01$. #,## Values significantly different from the Somatostatin $p < 0.05$, $p < 0.01$. +,+ +Values significantly different from the Curcumin $p < 0.05$, $p < 0.01$

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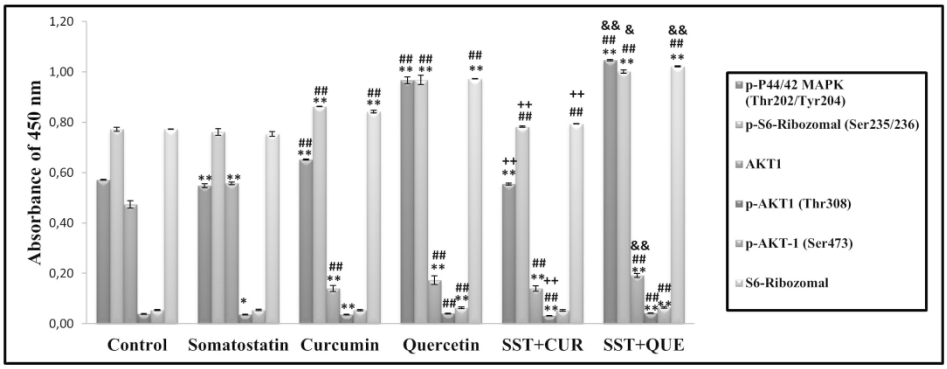

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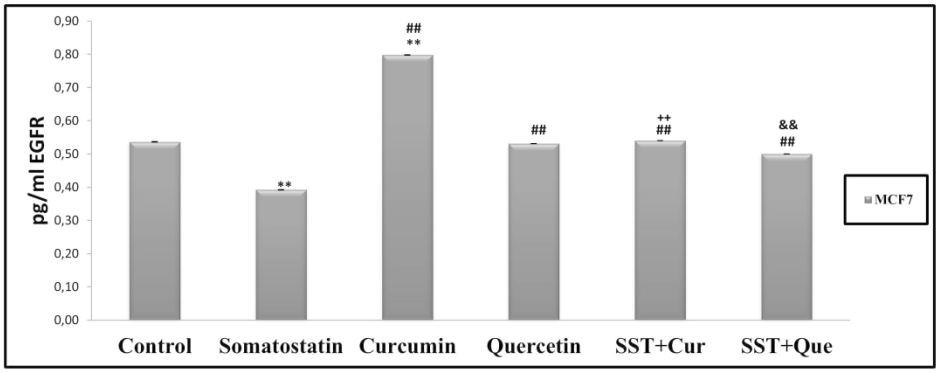

Figure 5: EGFR levels in MCF-7 cells after incubation for 24h with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin (n=6). .*,**Values significantly different from the control p <0.05, p < 0.01. &,&& Values significantly different from the Quercetin p<0.05, p <0.01. #,## Values significantly different from the Somatostatin p <0.05, p <0.01. +,+ +Values significantly different from the Curcumin p <0.05, p <0.01

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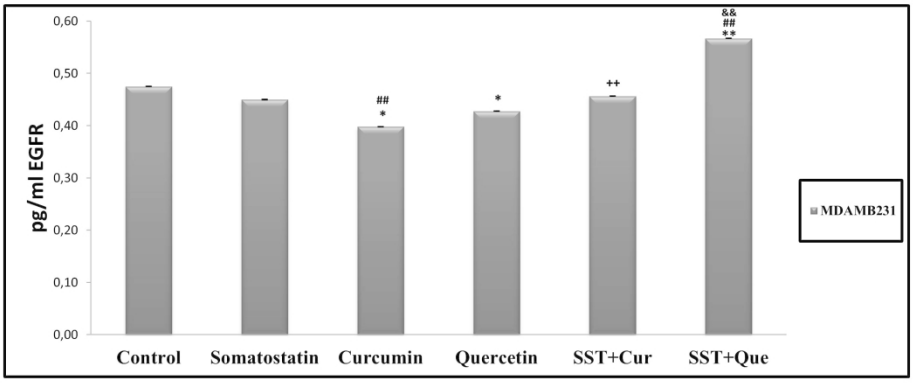

Figure 6: EGFR levels in MDA-MB231 cells after incubation for 24h with Somatostatin, Curcumin, Somatostatin+Curcumin, Quercetin or Somatostatin+Quercetin (n=6). **Values significantly different from the control p <0.01. .*,**Values significantly different from the control p <0.05, p < 0.01. &,&& Values significantly different from the Quercetin p<0.05, p <0.01. #,## Values significantly different from the Somatostatin p <0.05, p <0.01. +,+ +Values significantly different from the Curcumin p <0.05, p <0.01

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