Expression of somatostatin receptors subtypes (1-5) mRNA in MCF7 and MDA-MB231 breast cancer human cell lines

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Abstract

Background: breast cancer is one of most common types of cancer and heterogeneous disease. Somatostatin receptors (SSTR1-5) are expressed in breast cancer cells. MCF7 and MDA-MB231 cell lines used to evaluate the expression of somatostatin receptors (SSTRs 1 - 5).

Methods: The detection of mRNA expression levels of SSTRs in MCF7 and MDA-MB231 was performed using quantitative- polymerase chain reaction (qPCR).

Results: All SSTRs 1 - 5 were expressed in both cell lines. The SSTR 1, 2, 3 and 4 mRNA levels were significantly higher in MDA-MB-231 in relation to MCF-7. The expression of SSTR 4 and 5 mRNA was highest in MDA-MB231 and MCF-7 cell lines respectively, however, SSTR3 mRNA was least expressed in both cell lines.

Conclusion: All SSTRs were expressed in both MDA-MB231 and MCF7 cells. However, the levels of expression differ between both cell lines.

Keywords: Breast cancer. Cell lines .Somatostatin receptors. mRNA expression

Background

Breast cancer is the most common type of cancer and the second leading cause of cancer-related deaths in women worldwide [1]. Breast cancer is considered heterogeneous disease characterized by changes in the mammary epithelial cells leading to aggressive cell proliferation [2]. The three main biomarkers of interest in breast cancer include estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Currently, four molecular subtypes with different levels of expression of these receptors are luminal A, luminal B, HER2-enriched, and basal-like have been identified. Triple-negative breast cancer (TNBC), which is a part of the basal-like subgroup, it is characterized by the lack of PR, ER, and HER2 expression [3]. Different breast cancer subtypes displayed heterogeneity and extremely high mortality due to poor metastatic prognosis, drugs resistance and recurrent diseases [4]. At the most advanced stage of breast cancer, in particular the hormone-independent cancers develop resistance to therapy, thus causing increased mortality. The control and cure of breast cancer includes surgery, radiation therapy and chemotherapy as well as hormone therapy [5]. Several hormones are expressed in tumor tissues that lead to therapy resistance due to the biological actions of peptide hormones in regulating cellular process and proliferation [6]. Somatostatin (SST) is a widely expressed endogenous peptide known to inhibit the cellular processes such as neurotransmission, hormonal secretion and cell proliferation and differentiation as well as promoting apoptosis via five subtypes SSTRs (1-5) encoded by five distinct SSTR genes segregated on chromosomes 14, 16, 17, 20 and 22, respectively [7].

Somatostatin receptors (SSTRs) are G-protein-coupled plasma membrane receptors, initially secreted as a long precursor molecule; it undergoes specific enzymatic degradation generation with two forms of SST peptides, SS-14 and SS-28, as their natural ligands [8]. SSTRs1-5 expression is widely distributed in normal and tumor tissues depending on cell type. In normal tissues, SSTRs are found mainly in brain,

pancreas, stomach and kidney, and their expression varies in cancer cells depending on the tumor type and biological characteristics [9]. SSTRs have direct and indirect effects on tumor biology. The direct effect includes the inhibition of tumor proliferation and induction of pro-apoptotic pathways including intrinsic and extrinsic apoptosis pathway [10, 11]. Meanwhile, the indirect effects include the inhibition of hormones and growth factors that promote tumor growth [12, 13]. SSTRs mediate of signal transduction pathways through inhibition of adenylyl cyclase (AC) and guanylyl cyclase (GC), protein phosphorylation and activation of mitogen-activated protein kinase (MAPK)[14,15]. Furthermore, the activation of signaling pathways leads to the induction of cell cycle arrest via activation of cyclin dependent kinase inhibitor (p27Kip1) and apoptosis. Cancer cells with positive SSTRs are less malignant with higher survival rate whereas the lack of SSTRs expression has also been associated with poorly differentiated and invasive tumor. However, a number of effects demonstrated subtype selectivity, and subtype specific signaling has been reported [16]. SSTR1, 2, 4 and 5 frequently interfere with the mitogen-activate protein kinase pathway to modulate cell proliferation, whereas SSTR3 was indicated to have an increased potential to induce apoptosis [2]. In fact, SSTR2 is considered a good prognosis factor because it has association with low proliferative and invasive breast cancer. In addition, SSTRs being frequently expressed in the same cell, and the existence of ligand induced dimerization proposed for G protein coupled receptors, it is hypothesized that SSTRs are functionally excessive and act in concert [10]. Therefore, somatostatin analogues (SSAs) have been used in the treatment of SSTR-positive tumors [12]. However, the therapeutic results of SSAs treatment varied markedly due to the expression of SSTRs, different SSTR expression patterns and reasons that are not fully understood [17]. Expression levels of SSTRs have been determined in multiple human tumour tissues including neuroendocrine and non-endocrine tumor types like hepatocellular carcinoma, pancreatic cancer and breast cancer [18]. SSTRs are highly expressed in neuroendocrine tumors but their level of expression in breast cancer is not well documented [19]. In breast cancer, SSTRs are expressed in different levels and are correlated with various histological markers in a receptor-specific manner [20]. Previous studies have demonstrated the presence of SSTRs in a large variety of tumors and cancer cell lines [21]. In the present study, MCF-7 (estrogen-receptor positive breast cancer cell line) and MDA-MB-231 (estrogen-receptor negative breast cancer cell line) have been used in vitro as a breast cancer models to study the expression of these receptors. The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors. MDA-MB-231 is a highly aggressive, invasive but poorly differentiated triple-negative breast cancer (TNBC) cell line that lacks receptors for estrogen (ER) and progesterone (PR) expression, as well as HER2 (human epidermal growth factor receptor 2) amplification [22], and is known to be resistant to several anti-cancer agents [23]. The aim of this study was therefore to evaluate the mRNA expression for SSTRs (1-5) in human breast cancer cell lines MCF7 and MDA-MB231 using quantitative real-time polymerase chain reaction (qRT-PCR).

Materials And Methods

Cell culture

MCF7 and MDA-MB231 human breast cancer cell lines were kindly donated by the National Cancer Institute Regina Elena Rome, Italy. Both cells were purchased from the American Type Culture Collection (Manassas, VA, USA). MCF7 and MDA-MB231 cells were grown in a humidified 37 °C incubator in 5% CO₂ and cultured in D-MEM/F12 (Eagle's minimal essential medium /F12) complete media supplemented with 10% fetal bovine serum (FBS), 1% penicillin -streptomycin and 1% L-glutamine.

RNA Extraction

Total RNA from MCF7 and MDA-MB231 cell lines was extracted by using EXIQON kit. The concentrations of total RNA were quantified by Nano drop 2000 (Thermo Scientific, Hvidovre, Denmark), and all samples were stored at -20 °C until analysis.

Reverse-transcriptase Polymerase Chain Reaction (RT-PCR)

RNA was converted into complementary DNA (cDNA) by reverse transcriptase process according to manufacturer's instruction. After addition oligo (dT), samples were incubated at 42 °C for 1 hour in a thermal.

Quantitative Real -time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed using Bio-Rad Cyclor and Q Real-Time PCR Systems (Bio-Rad) to detect expression of SSTRs1-5 levels in MCF7 and MDA-MB231 breast cancer cell lines. qRT-PCR was performed using 2 µg of retro-transcribed RNA and normalized with GADPH. The quantity of mRNA relative to reference gene was calculated by $2^{-\Delta C_T}$ methods, analysis type is Single plex, and RQ min/max confidence level is 95.0. Samples were analysed using SYBR Green Super mix (Bio-Rad) according to manufacturer's instructions. The sample analysis was performed in triplicate and the experiments repeated three times in different batches of cell lines. Primer sequences used for SSTRs (1–5) are shown in Table 1. Table 1: Primer sequences used for mRNA SSTRs (1–5)

Table 1
Primer sequences used for mRNA SSTRs (1–5)

Receptor	Primer sequences
SSTR 1	Primer: 5'- CACATTTCTCATGGGCTTCCT-3' Reverse: 5'- ACAAACATCACCACCATC-3'
SSTR 2	Primer: 5'- GGCATGTTTGACTTTGTGGTG-3' Reverse: 5'- GTCTCATTCAGCCGGGATT-3'
SSTR 3	Primer: 5'- TGCCTTCTTTGGGCTCTACTT-3' Reverse: 5'- ATCCTCCTCCTCAGTCTTCTCC-3'
SSTR 4	Primer: 5'- CGTGGTCGTCTTTGTGCTCT-3' Reverse: 5'- AAGGATCGGCGGAAGTTGT-3'
SSTR 5	Primer: 5'- CTGGTGTTTGCGGGATGTT-3" Reverse: 5'- GAAGCTCTGGCGGAAGTTGT-3'

Statistical analysis

The SPSS software version 25 (IBM®) was used for the analysis. Data generated were expressed in means $\pm$ standard deviation. The mean expression of SSTRs levels in MCF7 and MDA-MB231 cell lines was compared using T-test. P-values of < 0.05 were considered statistically significant.

Results

SSTR1-5 mRNA expression was determined in both MCF-7 and MDA-MB-231 breast cancer human cell lines (Fig. 1) and the overall expression levels differ between the two cell lines. The SSTR 1, 2, 3 and 4 mRNA levels were significantly higher in MDA-MB-231 cell line in relation to MCF-7 cell line (P.value: 0.007, 0.001, < 0.001 and 0.003) respectively. The expression of SSTR4 mRNA was highest in MDA-MB231 cell line followed by SSTR2, SSTR1, SSTR5 and SSTR3 mRNA. In MCF7 cell line, SSTR4 has the highest expression levels, followed by SSTR1, SSTR5, SSTR2 and SSTR3. SSTR3 mRNA was least expressed in both cell lines, while SSTR4 has highly expressed in both cell lines too. An arbitrary score was adopted to summarize the expression levels of SSTRs as in (Table 2).

Table 2
Analysis of relative mRNA expression of SSTRs (1–5) in MCF7 and
MDA-MB231 cell lines

SSTRs Subtypes	MCF7	MDA-MB231
SSTR1	++	+++
SSTR2	++	+++
SSTR3	+	+
SSTR4	++	++++
SSTR5	++	++
Low level +, Moderate level ++, High level +++, Very high level++++		

Discussion

In this study, our data showed that all the SSTRs1-5 were expressed in both MCF-7 and MDA-MB-231 breast cancer cell lines. Similar findings have been reported that all the SSTR subtypes were expressed in both MCF7 and MDA-MB231 and SSTR3 mRNA being the least expressed in both cell lines, however, our somatostatin receptors' expression levels differ from those reported in the literature. SSTR4 was found to be express highly in MDA-MB231 cell lines while it is often reported that SSTR2 is predominant on breast cancer cells [24]. Many other studies were in agreement with the findings in the current study [5]. The expression of SSRT1, SSRT2, SSTR3 and SSTR4 were significantly higher in MDA-MB231 cell line [25]. According to the association between SSTRs and ER/PR positive receptors, it might be suggested that SSTR were overly expressed in MDA-MB231 cell lines that were correlated with poorly differentiated cancer cells. STTR1, 2, 3 and 4 have the potential role of blocking tumor growth by inhibiting cell cycle progression and inducing apoptosis. Furthermore, SSA antagonist may have more clinical benefits for ER and PR negative tumours. The highly expressed SSTR4 and low expressed SSTR3 was in agreement with earlier reports [26, 27]. The high expression of SSTR3 and SSTR2 or SSTR4 in MCF7 cell lines were linked to high apoptotic activity. Crucially, SST enhanced activation of cytotoxic signaling mainly mediated via SSTR2 and SSTR3 [28]. The low-level expression of SSTR3 has made it a target for breast cancer control or therapy. In light of this, activation of SSTR3 in MCF7 and MDA-MB231 breast cancer cell lines by SST agonist for cancer control is now been explored [29,]. However, the mechanism of SSTR3 in apoptosis and cell cycle arrest is still unclear [27]. Similarly, estrogen and progesterone receptors in MCF7 have been found to be important in breast cancer prognosis and development [30], and the positive estrogen effect on SSTR2 expression on regulation in breast cancer cells development has been documented. [31]. Furthermore, many of SST analogs have been synthesized for activation of single or multiple SSTRs while SST agonist are currently under development to control cancer cell proliferation [32, 33]. Several reports demonstrated that SSTRs expression in breast cancer is down regulated either in more aggressive and less differentiated tumors [34] or in anti-estrogen agents [35]. In this study, the high levels of SSTRs

expression were documented in aggressive tumors and thus, MDA-MB231 may be considered as target for therapeutic strategy. Meanwhile, the activation the expression levels of SSTR1, SSTR2, SSTR3 and or SSTR4 might enhance apoptotic activity in MCF7 cells. Several previous studies have investigated that SSTR expression may be able to be explored for further insights into conventional prognostic and therapeutic in breast cancer. In addition, the antiproliferative role of SST and its analogs have also been demonstrated [36]. Several in vitro studies have investigated the anti-proliferative effect of somatostatin analogs in breast cancer cells [36]. Previous studies have also shown that SSTR2 overexpression produces an anti-proliferative role in the estrogen-dependent MCF-7 cells by inducing apoptosis and decreasing EGFR expression [10]. Inhibition of tumor cell growth in MDA-MB-231 breast cancer cells using a somatostatin analogue associated with doxorubicin has been demonstrated [37]. Another study reported that a significant inhibition of growth in MDA-MB-231 and MCF-7 cells using a combination of somatostatin analogs and doxorubicin. Studies also suggested that the use of somatostatin analogs could induce positive regulation of SSTRs expression and thought to block tumor progression. These results highlighted the SSTRs -targeted therapy in which the evaluation that SSTR1-5 is expressed in both breast cancer cell lines MCF7 and MDA-MB231. These findings also recommended furthering understand the anti-proliferative role of SSTRs in breast tumor biology to improve therapy and diagnostic processes in estrogen receptors positive and estrogen receptors negative breast cancers.

Conclusion

SSTRs (1–5) were expressed in both MDA-MB231 and MCF7 cell lines, but the level of expression differed between both cell lines. The activation of SSTRs receptors in ER+, PR + tumor may be considered. SSTRs overexpression in aggressive tumors (ER-, PR-) may be considered as target for therapeutic strategy. Future research is warranted to study the functions of SSTRs.

Abbreviations

SSTRs: Somatostatin receptors; qRT-PCR: Quantitative- polymerase chain reaction; mRNA: Messenger RNA; ER: Estrogen receptors; PR: Progesterone receptors.

Declarations

Acknowledgments

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Author contributions

Design and performed the experiments: Alkhansa M, Zuki A, Hazilawati H, Mohd H. Contributed reagents/materials/analysis tools: Maria Tereasa M. Analyzed the data: Alkhansa M and Barbara T. All

authors involved in the study performed, data analysis, results interpretation, manuscript drafting or manuscript revision, manuscript editing, and approval of final manuscript submitted.

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A viability of data and materials

All data analyzed during this study are included in this published article.

Ethics approval and consent for participants

Not applicable

Competing interests

The authors declare that they haven't Competing interests.

Figures

Figure 1

mRNA expression levels of SSTRs (1-5) in human breast cancer cell lines. NS: No significant, **: $p < 0.01$, ***: $p < 0.001$.