CHAPTER 7

α1-ADRENOCEPTOR AGONISTS INFLUENCE BREAST CANCER CELL SURVIVAL AND PRO-ANGIOGENIC AND SIGNALING MOLECULE EXPRESSION DEPENDING ON ESTROGEN RECEPTOR (ER) STATUS UNLIKE α2-AR AGONISTS

Specific Objective 2A: Involvement of Cellular Mechanisms in α-AR-induced effects on human breast cancer cells.

7.1 Rationale:

Dysregulation in neuroendocrine-immune functions, increased sympathetic activity in stress associated with chronic diseases, the presence of multiple adrenergic receptor subtypes on cancer cells and the scope of how altered hormone sensitivity may influence downstream signaling pathways triggered by adrenergic agonists have not been investigated.

Previously, we have reported that reproductive aging is characterized by alterations in hypothalamic catecholaminergic activity, decline in sympathetic noradrenergic innervation in the lymphoid organs and associated immunosuppression which may contribute to development of age-related diseases and cancer. Development and progression of hormone-responsive cancers in females may be partly mediated
through ER-regulation of $\alpha$-adrenoceptor activity. Hence, the present study aims to investigate the role of alpha-adrenoceptors ($\alpha$-AR) in modulating proliferation and expression of pro-angiogenic factors, and signaling molecules in ER (+) and ER (-) breast cancer cell lines.

7.2 Methods:

7.2.1 Culture:

7.2.1.1 MCF-7 cell line

MCF-7 human breast cancer cell line was obtained from NCCS, Pune and maintained in DMEM medium, supplemented with 2mM L-Glutamine, 100 units/ml Penicillin, 100 $\mu$g/ml Streptomycin, 1.5 g/l sodium bicarbonate and 10% Fetal Bovine Serum and incubated at 37°C in a humidified atmosphere with 5% CO$_2$.

7.2.1.2 MDA MB-231 cell line

MDA MB-231 human breast cancer cell lines were obtained from NCCS, Pune and maintained in L15 medium, supplemented with 2mM L-Glutamine, 100 units/ml Penicillin, 100 $\mu$g/ml Streptomycin, 1.5 g/l sodium bicarbonate and 10% Fetal Bovine Serum and incubated at 37°C in a humidified atmosphere without CO$_2$.

Cells were cultured until they reach 70% confluence and such sub confluent flasks were tripsinised and seeded in 96 well plates (5000 cells/well) and incubated for 24 hours until the monolayer is formed. (Cells were stained using trypan blue, live cells were counted by the exclusion method and the desired cell number was achieved for plating onto 96 well plates.)

7.2.2 Treatment

7.2.2.1 Experiment 1

Cells were incubated with $10^{-3}$ M, $10^{-6}$ M and $10^{-9}$ M phenylephrine (alpha 1 adrenergic agonist) with and without $10^{-5}$ M prazosin ($\alpha_1$-AR-specific antagonist) for 2, 4 and 6 days. After 2, 4 and 6 days, MTT assay was performed to measure cellular proliferation. Supernatants were collected and stored for measurement of VEGF A and VEGF C production using ELISA and nitric oxide production using Greiss reagent system.
as described in Chapter 2. Cell pellets were collected after 2, 4 and 6 days of treatment with the agonists and antagonists, lysed using RIPA buffer and the expression of p-ERK, p-CREB and p-Akt were measured in the lysates using ELISA.

7.2.2.2 Experiment 2

Cells were incubated with $10^{-3}$ M, $10^{-6}$ M and $10^{-9}$ M Clonidine (alpha 2 adrenergic agonist) with and without 10 µM idazoxan ($\alpha_2$-AR-specific antagonist) for 2, 4 and 6 days. After 2, 4 and 6 days, MTT assay was performed to measure cellular proliferation. Supernatants were collected and stored for measurement of VEGF A and VEGF C production using ELISA and nitric oxide production using Greiss reagent system as described in Chapter 2. Cell pellets were collected after 2, 4 and 6 days of treatment with the agonists and antagonists, lysed using RIPA buffer and the expression of p-ERK, p-CREB and p-Akt were measured in the lysates using ELISA.

7.3 Results

7.3.1 $\alpha_1$-AR agonist phenylephrine selectively enhances the proliferation of ER (+) breast cancer cell line MCF-7 alone

The proliferation of ER (+) cell line MCF-7 was significantly ($p<0.05$) enhanced by treatment with $\alpha_1$-AR agonist phenylephrine ($10^{-9}$ M and $10^{-6}$ M) for 2 days (Fig. 7.1A). The $\alpha_1$-AR agonist-mediated increase in the proliferation of ER (+) MCF-7 cells was sustained after 4 and 6 days of incubation and the effects were reversed by co-treatment with the $\alpha_1$-AR-specific antagonist prazosin on the 4th day alone ($p<0.05$; Fig. 7.1B).

Interestingly, treatment with prazosin ($10^{-5}$ M) alone significantly decreased proliferation of ER (+) MCF-7 cells.

Contrary to its effects on ER (+) breast cancer cells, the $\alpha_1$-AR agonist phenylephrine did not significantly affect the proliferation of ER (-) MDA MB-231 cells in vitro after 2, 4 and 6 days of treatment (Fig. 7.1C and Fig. 7.1D).
Fig. 7.1  

_In vitro addition of α₁-AR agonist phenylephrine on proliferation of ER (+) MCF-7 and ER (-) MDA MB-231 breast cancer cell lines._

Co-incubation with α₁-AR agonist phenylephrine enhanced proliferation for 2, 4 and 6 days of incubation and the effects were reversed by co-treatment with the antagonist prazosin on the 4th day alone (Fig. 1A, 1B) in ER (+) MCF-7 cells but not ER (-) MDA MB-231 cells (1C, 1D). *p<0.05 from control; #p<0.05 from agonist-treated group.
7.3.2 $\alpha_1$-AR agonist upregulates expression of VEGF A and downregulates VEGF C in ER (+) MCF-7 cells

There was a marginal increase in the expression of VEGF A by ER (+) MCF-7 cells upon stimulation of $\alpha_1$-AR using specific agonist phenylephrine ($10^{-3}$ M, $10^{-6}$ M and $10^{-9}$ M) and the effects were reversed upon co-incubation with $\alpha_1$-AR antagonist prazosin ($10^{-5}$ M) after 2 days of treatment (Fig. 7.2A). However, the effects of phenylephrine ($10^{-6}$ M) on VEGF A production were not sustained after 4 and 6 days of incubation although the $\alpha_1$-AR blocker prazosin consistently decreased VEGF A expression by MCF-7 cells up to 6 days (Fig. 7.2B). Interestingly, treatment with prazosin ($10^{-5}$ M) alone significantly decreased VEGF A production by ER (+) MCF-7 cells after 2, 4 and 6 days of treatment.

Treatment with phenylephrine ($10^{-3}$ M, $10^{-6}$ M and $10^{-9}$ M) significantly decreased the production of VEGF C by MCF-7 cells after 2 days of treatment and the effect was not reversed by co-treatment with the antagonist (Fig. 7.2C). Similar to VEGF A, effects of phenylephrine ($10^{-6}$ M) on VEGF C expression were not sustained after 4 and 6 days of incubation (Fig. 7.2D).

7.3.3 $\alpha_1$-AR agonist downregulates expression of VEGF A and upregulates VEGF C in ER (-) MDA MB-231 cells

Stimulation of $\alpha_1$-AR on ER (-) MDA MB-231 cells using specific agonist phenylephrine did not alter expression of VEGF A after two days of treatment (Fig. 7.3A). However, prolonged treatment with $\alpha_1$-AR agonist phenylephrine ($10^{-6}$ M) for 6 days significantly decreased the production of VEGF A by MDA MB-231 cells and the effects were reversed by co-treatment with $\alpha_1$-AR-specific antagonist prazosin ($10^{-5}$ M; Fig. 7.3B).

VEGF C production by ER (-) MDA MB-231 cells was also not significantly altered after 2 days of treatment with $\alpha_1$-AR agonist phenylephrine (Fig. 7.3C). However, after 6 days of treatment, the $\alpha_1$-AR agonist significantly enhanced production of VEGF C by MDA MB-231 cells, although the effect was not reversed by co-treatment with the antagonist (Fig. 7.3D).
**Fig. 7.2** Differential expression of VEGF A and C in ER (+) MCF-7 cells upon $\alpha_1$-AR stimulation using phenylephrine. VEGF A expression was increased (2A, 2B) while VEGF C was significantly decreased (2C, 2D) upon treatment with phenylephrine for 2 days alone. $\alpha_1$-AR antagonist prazosin co-treatment reversed the effects on VEGF A alone. *p<0.01 from control; #p<0.05 from agonist-treated group.
Fig. 7.3  Contrary expression of VEGF A and C in ER (-) MDA MB-231 cells upon \( \alpha_1 \)-AR stimulation with phenylephrine. Prolonged treatment of ER (-) MDA MB-231 cells with phenylephrine for 6 days significantly decreased VEGF A expression (3A, 3B) and enhanced production of VEGF C (3C, 3D) in vitro. Both VEGF A and VEGF C expression were not reversed upon co-treatment with the antagonist. *p<0.05 from control; #p<0.05 from agonist-treated group.
7.3.4 $\alpha_1$-AR agonist differentially modulates nitric oxide production by ER (+/-) breast cancer cells

$\alpha_1$-AR agonist phenylephrine ($10^{-6}$ M and $10^{-9}$ M) treatment significantly enhanced the production of NO by MCF-7 cells after 4 days of treatment although co-treatment with antagonist did not reverse the agonist-mediated increase (Fig. 7.4A).

Prolonged treatment of MCF-7 cells with $\alpha_1$-AR agonist phenylephrine significantly enhanced nitric oxide production up to 6 days of treatment (Fig. 7.4B).
Stimulation of α₁-AR on MDA MB-231 cells on the other hand significantly decreased nitric oxide production in the highest dose alone (Phe 10⁻³ M) after 4 days of incubation (Fig. 7.4C). Prolonged incubation of phenylephrine 10⁻⁶ M with MDA MB-231 cells for 6 days did not significantly alter nitric oxide production (Fig. 7.4D).

**Fig. 7.4** α₁-AR stimulation differentially regulate nitric oxide production in ER (+) MCF-7 cells and ER (-) MDA MB-231 cells. While α₁-AR agonist phenylephrine enhanced the production of NO by MCF-7 cells (A), it decreased the production of NO by MDA MB-231 cells (B). Effects on ER (+) MCF-7 cells were sustained up to 6 days of treatment (C) although in ER (-) MDA MB-231 cells the effects were not sustained (D). *p<0.05 from control; #p<0.05 from agonist-treated group.

### 7.3.5 α₁-AR stimulation of ER (+) cells enhances phosphorylation of ERK, CREB and Akt in vitro

Expression of p-ERK was significantly enhanced in ER (+) MCF-7 cells treated with phenylephrine (10⁻⁶ M and 10⁻⁹ M) and decreased upon co-incubation of phenylephrine (10⁻³ M) treated cells with the specific antagonist prazosin (10⁻⁵ M) after 2 days of incubation (Fig. 7.5A).

The effects of phenylephrine on p-ERK expression were sustained after prolonged incubation of MCF-7 cells with the agonist (10⁻⁶ M) for 4 and 6 days. Co-treatment with the antagonist reversed the agonist-mediated increase in p-ERK expression only after 6 days of treatment (Fig. 7.5B). Interestingly, co-incubation of MCF-7 cells with the α₁-AR antagonist prazosin (10⁻⁵ M) alone also significantly enhanced p-ERK expression after 2 and 4 days of treatment (Fig. 7.5A, 7.5B). p-CREB expression was however significantly increased in cells treated with 10⁻⁶ M of phenylephrine and significantly decreased on co-incubation with the higher dose (10⁻³ M) after 2 days of incubation. The agonist-mediated increase in p-CREB expression was significantly reversed by co-treatment with the α₁-AR antagonist prazosin (Fig. 7.5 C).

Phenylephrine (10⁻⁶ M) mediated increase in p-CREB expression was sustained after 4 days of treatment although the effect was not reversed after 4 days (Fig. 7.5D). Interestingly, treatment of MCF-7 cells with α₁-AR antagonist prazosin alone significantly decreased p-CREB expression after 2, 4 and 6 days of incubation (Fig. 7.5C, 7.5D).
Treatment of ER (+) MCF-7 cells with phenylephrine (10^{-9} M) significantly enhanced p-Akt expression after 2 days of incubation (Fig. 7.5E). Prolonged treatment with phenylephrine (10^{-6} M) also significantly enhanced p-Akt expression after 4 days of incubation (Fig. 7.5E). Co-incubation with antagonist prazosin, significantly reversed the agonist-mediated effect on p-Akt expression after 6 days of treatment (Fig. 7.5F).

Interestingly, treatment of ER (+) MCF-7 cells with α_{1}-AR antagonist prazosin alone also significantly enhanced p-Akt expression after 2 and 4 days of incubation (Fig. 7.5E, 7.5F).

**Fig. 7.5** *In vitro effects of α_{1}-AR signaling in ER (+) cells are mediated through p-ERK, p-CREB and p-Akt.* Phenylephrine treatment of ER (+) MCF-7 cells significantly enhanced p-ERK (A, B), p-CREB (C, D) and p-Akt expression (E, F) and the effects were reversed by co-treatment with the α_{1}-AR antagonist prazosin. *p<0.05 from control; #p<0.01 from agonist-treated group.

### 7.3.6 α_{1}-AR agonist phenylephrine decreases p-Akt expression in ER (-) MDA MB-231 cells

α_{1}-AR agonist phenylephrine did not significantly alter p-ERK expression of ER (-) cells in the presence and absence of specific antagonist prazosin after 2, 4 and 6 days of treatment (Fig. 7.6A, 7.6B). Similarly, p-CREB expression was also not significantly altered by treatment of α_{1}-AR agonist phenylephrine in ER (-) MDA MB-231 cells co-treated with or without prazosin (Fig. 7.6C, 7.6D).

p-Akt expression however, was significantly decreased in MDA MB-231 cells treated with phenylephrine (10^{-3} M) and the effect was reversed after co-treatment with the antagonist prazosin (10^{-5} M; Fig. 7E). Prolonged treatment of phenylephrine (10^{-6} M) with MDA MB-231 cells also significantly decreased the p-Akt expression after 4 days of incubation although the effect was not reversed by co-treatment with the antagonist (Fig. 7F).

Contrary to ER (+) cell line MCF-7, co-incubation of ER (-) MDA MB-231 cells with the α_{1}-AR antagonist prazosin alone did not significantly affect p-ERK, p-CREB or p-Akt expression *in vitro.*
Fig. 7.6  *In vitro effects of α₁-AR signaling in ER (-) MDA MB-231 cells are mediated through down regulation of p-Akt.*  α₁-AR agonist phenylephrine did not significantly alter p-ERK (A, B) and p-CREB (C, D) expression of ER (-) MDA MB-231 cells although p-Akt expression (E, F) was significantly decreased and the effect was reversed after co-treatment with the antagonist prazosin. *p<0.05 from control; #p<0.05 from agonist-treated group.

**7.3.7 α₂-AR agonist clonidine decreases proliferation of ER (+) MCF-7 cells and enhances ER (-) MDA MB-231 cell proliferation**

The proliferation of ER (+) cell line MCF-7 was significantly (p<0.05) increased by treatment with α₂-AR agonist clonidine (10⁻³ M) for 2 days and the effects were reversed by co-incubation of these cells with the α₂-AR-specific antagonist idazoxan (10⁻⁵ M; Fig. 7.7A). The α₂-AR agonist-mediated increase in the proliferation of ER (+) MCF-7 cells was sustained up to 4 days of incubation (Fig. 7.7B). Treatment of MCF-7 cells with α₂-AR antagonist idazoxan alone significantly decreased proliferation after 2 days (Fig. 7.7A, 7.7B).

Similarly, the α₂-AR agonist clonidine (10⁻³ M) significantly enhanced the proliferation of ER (-) MDA MB-231 cells in *vitro* after 2 days of treatment (7.7C) Upon prolonged incubation of 10⁻⁶ M clonidine the increase in proliferation was sustained for 6 days of treatment (Fig. 7.7D). Co-incubation of MDA MB-231 cells treated with clonidine (10⁻⁶ M) with α₂-AR antagonist idazoxan significantly reversed the agonist-mediated increase in proliferation after 4 days of incubation.

**7.3.8 α₂-AR agonist selectively downregulates VEGF C production in ER (+) MCF-7 cells**

There was no significant effect of α₂-AR agonist clonidine treatment on the VEGF A production by MCF-7 cells after 2, 4 and 6 days of treatment (Fig. 7.8A, 7.8B). However, VEGF C production was significantly decreased after 2 days of incubation with α₂-AR agonist clonidine which was not reversed by co-treatment with the α₂-AR-specific antagonist Idazoxan (Fig. 7.8C).

Prolonged incubation of MCF-7 cells with clonidine (10⁻⁶ M) for 4 and 6 days did not affect VEGF-C production (Fig. 7.8D). Interestingly, VEGF C production was also
significantly decreased by treatment of MCF-7 cells with $\alpha_2$-AR-specific antagonist Idazoxan for 2 and 4 days (Fig. 7.8C, 7.8D).

Fig. 7.7 $\alpha_2$-AR stimulation decreases proliferation of ER (+) MCF-7 cells and enhances ER (-) MDA MB-231 cell proliferation. The proliferation of ER (+) cell line MCF-7 (A, B) and ER (-) cell line MDA MB-231 (C, D) was increased by treatment with $\alpha_2$-AR agonist clonidine and reversed by co-treatment with $\alpha_2$-AR-specific antagonist idazoxan. *p<0.05 from control; #p<0.05 from agonist-treated group.
VEGF A was unaltered while VEGF C was decreased by α₂-AR agonist in ER (+) MCF-7 cells. VEGF A production by MCF-7 cells was not altered after treatment with α₂-AR agonist clonidine (A, B). There was a decrease in VEGF C production after incubation with α₂-AR agonist clonidine and α₂-AR-specific antagonist Idazoxan (C, D). *p<0.01 from control.
7.3.9 $\alpha_2$-AR agonist enhances VEGF A and VEGF C production in ER (-) MDA MB-231 cells

There was a significant increase in VEGF A production by ER (-) MDA MB-231 cells after 4 days of incubation with $\alpha_2$-AR-specific agonist clonidine ($10^{-6}$ M) although the effects were not reversed by co-treatment with the $\alpha_2$-AR-specific antagonist idazoxan ($10^{-5}$ M; Fig. 7.9A, Fig. 7.9B).

Similarly, VEGF C production by ER (-) MDA MB-231 cells was significantly enhanced upon co-treatment with $\alpha_2$-AR agonist clonidine ($10^{-6}$ M) for 4 days and cells co-incubated with $\alpha_2$-AR-specific antagonist idazoxan and clonidine showed significantly decreased VEGF C expression after 2 days of treatment compared with agonist-treated group (Fig. 7.9C; Fig. 7.9D).

7.3.10 $\alpha_2$-AR agonist decreases nitric oxide production in ER (+) MCF-7 and ER (-) MDA MB-231 cells

There was a significant decrease in nitric oxide production by ER (+) MCF-7 cells upon co-treatment with $\alpha_2$-AR-specific agonist clonidine ($10^{-3}$ M, $10^{-6}$ M, $10^{-9}$ M) after 2 days of incubation (Fig. 7.10A). The effects of the agonist ($10^{-9}$ M) alone were reversed by co-treatment with $\alpha_2$-AR-specific antagonist idazoxan ($10^{-5}$ M). Prolonged incubation of ER (+) MCF-7 cells with the agonist alone for 4 and 6 days also decreased nitric oxide production while co-incubation with the antagonist reversed it (Fig. 7.10 B). Interestingly, co-treatment of MCF-7 cells with $\alpha_2$-AR antagonist idazoxan alone also significantly decrease nitric oxide production. In ER (-) cells treatment with $\alpha_2$-AR agonist clonidine significantly decreased nitric oxide production (Clo $10^{-6}$ M and $10^{-3}$ M; Fig. 7.10C). Co-treatment of clonidine-treated MDA MB-231 cells with $\alpha_2$-AR-specific antagonist idazoxan however, significantly enhanced nitric oxide production. The effects of the agonist (Clo $10^{-6}$ M) in vitro were sustained up to 6 days of treatment and reversed upon co-treatment with the antagonist until the 4th day (Fig. 7.10D). Interestingly, $\alpha_2$-AR blockade using idazoxan alone significantly enhanced nitric oxide production in ER (-) MDA MB-231 cells after 2 and 4 days of treatment.
**Fig. 7.9**  
*VEGF A and VEGF C production were not altered in ER (-) MDA MB-231 cells.* Treatment with clonidine did not alter VEGF A (A, B) and VEGF C (C, D) production by ER (-) MDA MB-231 cells and co-incubation with α2-AR-specific antagonist idazoxan enhanced it.*p<0.05 from control; #p<0.05 from agonist-treated group.*
**Fig. 10**  
Nitric oxide production by ER (+) MCF-7 and ER (-) MDA MB-231 cells was decreased by α₂-AR agonist. Nitric oxide production by ER (+) MCF-7 cells (A, B) and ER (-) MDA MB-231 cells (C, D) was decreased by clonidine treatment and reversed by co-treatment with α₂-AR-specific antagonist idazoxan. Although idazoxan treatment alone decreased nitric oxide production in ER (+) MCF-7 cells, it significantly enhanced nitric oxide production in ER (-) MDA MB-231 cells. *p<0.05 from control; #p<0.05 from agonist-treated group.
7.3.11 α2-AR agonist selectively decreases p-CREB expression in ER (+) MCF-7 cells

p-ERK expression by ER (+) MCF-7 cells was not significantly altered by incubation with α2-AR agonist clonidine for 2, 4 and 6 days (Fig. 7.11A, 7.11B). However, p-CREB expression significantly declined upon co-incubation of MCF-7 cells with clonidine (10⁻³ M, 10⁻⁶ M, 10⁻⁹ M; Fig. 7.11C). Co-incubation with α2-AR-specific antagonist idazoxan did not reverse the clonidine (10⁻⁶ M)-mediated decline p-CREB expression by MCF-7 cells. Increasing the incubation time to 4 and 6 days also showed similar results (Fig. 7.11D). Interestingly, treatment of MCF-7 cells with the α2-AR antagonist idazoxan alone also significantly decreased p-CREB expression after 2, 4 and 6 days of treatment. p-Akt expression on the other hand was not significantly altered upon treatment of MCF-7 cells with α2-AR agonist clonidine in the presence and absence of specific antagonist idazoxan for 2, 4 and 6 days (Fig. 7.11E, 7.11F).

7.3.12 α2-AR agonist decreases p-CREB and p-Akt expression in ER (-) MDA MB-231 cells while antagonist increases p-ERK expression

There was no significant effect of the α2-AR agonist clonidine on p-ERK expression by ER (-) MDA MB-231 cells after 2, 4 and 6 days of treatment. Interestingly, treatment of MDA MB-231 cells with α2-AR antagonist idazoxan (10⁻⁵ M) alone significantly enhances p-ERK expression after 2 days of treatment alone (Figs. 7.12A, 7.12B). p-CREB expression however was significantly decreased upon co-incubation of ER (-) MDA MB-231 cells with α2-AR agonist clonidine (10⁻³ M, 10⁻⁶ M and 10⁻⁹ M) after 2 days of treatment. The agonist-mediated decline was not reversed by co-treatment with the antagonist. Also, the effects were not sustained after prolonged incubation with the agonist for 4 and 6 days (Figs. 7.12C, 7.12D). Finally, p-Akt expression was significantly decreased by co-treatment of ER (-) cells with α2-AR agonist clonidine for 2 days. The effects were not reversed by co-treatment with the antagonist and not sustained beyond 2 days of treatment (Figs. 7.12E, 7.12F).

Fig. 7.11  Selective down regulation of p-CREB signaling by α2-AR agonist in ER (+) MCF-7 cells. p-ERK (11A, 11B) and p-Akt (11E, 11F) expression by ER (+) MCF-7 cells was not significantly altered by incubation with α2-AR agonist clonidine. p-CREB expression was down regulated by clonidine and idazoxan treatment (11C, 11D). *p<0.01 from control.
Fig. 7.12  Decreased p-CREB and p-Akt signaling by α2-AR stimulation and increased p-ERK signaling by α2-AR blockade in ER (-) MDA MB-231 cells. Although α2-AR agonist clonidine did not alter p-ERK expression (A) by ER (-) MDA MB-231, it significantly decreased p-CREB (12B) and p-Akt (12C) expression which were not reversed by co-treatment with the antagonist. α2-AR blockade by idazoxan enhanced p-ERK expression alone. *p<0.05 from control.

7.4 Key Findings

Proliferation, VEGF A and NO production were enhanced in ER (+) MCF-7 cells treated with α1-AR agonist while VEGF C expression alone was enhanced in ER (-) MDA MB-231 cells. α1-AR agonist enhanced the expression of p-ERK, p-CREB and p-Akt in ER (+) MCF-7 cells alone.

Treatment of MCF-7 and MDA MB-231 cells with α2-AR agonist similarly enhanced proliferation and decreased NO production and p-CREB expression. VEGF C expression was decreased in ER (+) MCF-7 cells while p-Akt expression was decreased in ER (-) MDA MB-231 cells.

α1-AR blockade reversed proliferation and VEGF A production by ER (+) MCF-7 cells while α2-AR blockade reversed the proliferation of MCF-7 and MDA MB-231 cells and VEGF C production by MCF-7 cells.

These results suggest that the survival signaling and angiogenesis in cancer cells are selectively modulated depending upon the presence of the estrogen receptor and subtype of α-AR. Such α-AR-dependent actions ER (+) and ER (-) cells are mediated through selective alteration in the intracellular signaling pathways involving ERK, CREB and Akt mediated by pro-angiogenic signals (VEGF A, C and NO). Taken together, development and progression of hormone-responsive cancers in females may be partly mediated through ER-regulation of α-adrenoceptor activity.