HHS Public Access

Author manuscript

Biochem Biophys Res Commun. Author manuscript; available in PMC 2020 February 05.

Published in final edited form as:

Biochem Biophys Res Commun. 2019 February 05; 509(2): 476–482. doi:10.1016/j.bbrc.2018.12.145.

Overexpression of the steroidogenic acute regulatory protein in breast cancer: Regulation by histone deacetylase inhibition

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Abstract

Dysregulation of steroid hormone biosynthesis has been implicated in the pathophysiology of a variety of cancers. One such common malignancy in women is breast cancer that is frequently promoted by estrogen overproduction. All steroid hormones are made from cholesterol, and the rate-limiting step in steroid biosynthesis is primarily mediated by the steroidogenic acute regulatory (StAR) protein. Whereas the involvement of StAR in the regulation steroid hormone biosynthesis is well established, its association to breast cancer remains obscure. Herein, we report that estrogen receptor positive breast cancer cell lines (MCF7, MDA-MB-361, and T-47D) displayed aberrant high expression of the StAR protein, concomitant with 17β-estradiol (E2) synthesis, when compared their levels with normal mammary epithelial (MCF10A and MCF12F) and triple negative breast cancer (MDA-MB-468, MDA-MB-231, and BT-549) cells. StAR was identified as a novel acetylated protein in MCF7 cells, in which liquid chromatography-tandem mass spectrometry analysis identified seven StAR acetyl lysine residues under basal and in response to histone deacetylase (HDAC) inhibition. A number of HDAC inhibitors were capable of diminishing StAR expression and E2 synthesis in MCF7 cells. The validity of StAR protein acetylation and its correlation to HDAC inhibition mediated steroid synthesis was demonstrated in adrenocortical tumor H295R cells. These findings provide novel insights that StAR protein is abundantly expressed in the most prevalent hormone sensitive breast cancer subtype, wherein inhibition of HDACs altered StAR acetylation patterns and decreased E2 levels, which may have important therapeutic implications in the prevention and treatment of this devastating disease.

Keywords

Breast cancer; StAR; steroid biosynthesis; estrogen; acetylation; HDAC inhibition

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Conflicts of Interest

The authors have nothing to declare.

1. Introduction

Steroid hormones play crucial roles in diverse processes, ranging from development to diseases. The steroidogenic acute regulatory protein (StAR; also called STARD1) primarily mediates the biosynthesis of steroid hormones, by controlling the transport of the substrate of all steroid hormones, cholesterol, from the outer to the inner mitochondrial membrane [1,2]. In fact, regulation of StAR expression, thus, steroid biosynthesis, is a key event to appropriate functioning of a variety of cholesterol/steroid led activities. It has been shown that agents that influence StAR expression also influence steroid synthesis by mechanisms that enhance transcription, translation, or activity of StAR [2,3]. Conspicuously, whereas StAR's gain-of-function is associated with the optimal cholesterol transferring ability of the StAR protein in steroid biosynthesis, its loss-of-function profoundly affects the steroidogenic response. Accordingly, the StAR protein plays an indispensable role in the regulation of steroid hormone biosynthesis in a variety of classical and non-classical steroidogenic tissues [2,4]. It is noteworthy that malfunction in the steroidogenic machinery, involving androgen and/or estrogen biosynthesis, has been implicated in the pathogenesis of a variety of malignancies [5,6].

A substantial number of cancers, including breast, are hormone sensitive and evolve due to dysregulation in steroid biosynthesis. Breast cancer, the most prevalent form of cancer in women globally, is activated by estrogens, especially 17β-estradiol (E2), and it accounts for over one-fourth of all cancer cases [5-7]. Hormone sensitive breast cancers majorly express estrogen receptor (ER), especially ERα and/or progesterone receptor (PR), and account for ~80% of all breast cancer cases. Conversely, 15-20% of breast cancers lack ER and PR expression. These cases are categorized as human epidermal growth factor receptor 2/the erythroblastosis oncogene-B2 positive (HER2/ErbB2+) when they express HER2, while the remaining cases that do not express ER, PR, and HER are called triple negative breast cancer (TNBC) [8,9]. Regardless of cancer types, the majority of breast cancer tumors express high levels of aromatase (the key enzyme in estrogen biosynthesis), concomitant with large amounts of estrogens, in the development and growth of cancers. Consequently, suppression of estrogen synthesis by blocking aromatase and/or inhibition of ERα activation are considered as effective endocrine therapies in breast cancer treatment [10-12]. However, endocrine therapies develop many undesirable side effects by diminishing whole body estrogens, in addition to resistance that is the leading cause of cancer death, warranting additional appropriate strategies.

An abnormal epigenetic control is a common early event in tumorigenesis. Histone deacetylases (HDACs) regulate many important cellular processes, including chromatin remodeling, cell signaling, and genomic stability through the dynamic process of deacetylation of histone and non-histone proteins [13,14]. HDACs are frequently dysregulated in various cancers. HDAC inhibitors are a novel category of anti-cancer drugs that modulate acetylation by targeting histone deacetylases. Inhibition of HDACs interferes with protein deacetylation and alters biological processes, including cell proliferation, cell cycle arrest, and apoptosis in tumor/cancer cells. Given the importance of the StAR protein in the regulation of steroid biosynthesis, the correlation between HDAC inhibition and StAR mediated estrogen production within the context of breast cancer is of a major therapeutic

interest. The present studies demonstrate for the first time that StAR protein is highly expressed in ER+ breast cancer in which it is acetylated, and inhibition of HDACs alters StAR's acetylation patterns affecting E2 levels in MCF7 cells.

2. Materials and methods

2.1. Cell Cultures and Reagents

Human normal mammary epithelial MCF10A (CRL-10317) and MCF12F (CRL-10783) cells, and ER+ MCF7 (HTB-22), MDA-MB-361 (HTB-27), and T-47D (HTB-133), and triple negative MDA-MB-468 (HTB-132), BT-549 (HTB-19), and MDA-MB-231 (HTB-26) breast cancer cells were purchased from ATCC (Manassas, VA), and were maintained in specific growth media containing antibiotics [15], according to instructions from the ATCC. Human adrenocortical tumor H295R (CRL-2128) cell line was obtained from ATCC and were cultured in DMEM/F12 with 1% ITS plus 2.5% NuSerum containing penicillin/streptomycin (Invitrogen, Carlsbad, CA) [16].

LBH589 (Panobinostat), MS-275 (Entinostat), and SB939 (Practinostat) were purchased from APExBIO (Houston, TX), and Vorinostat (SAHA), Sirtuin (SIRT) 1/2 inhibitors IV and VII, and romidepsin (Istodax, FR228) were purchased from Millipore-Sigma (St. Louis, MO). StAR (Ab133657 or Ab180804; AbCam, Cambridge, MA), aromatase (Ab124776, AbCam), and acetyl lysine (05-515, AbCam), and β-actin (sc47778, Santa Cruz Biotechnology) antibodies (Abs) were purchased from the indicated commercial sources.

2.2. Immunoblotting

Western blotting (WB) studies were carried out using total cellular protein. Briefly, cells were washed, homogenized in RIPA lysis buffer (25mM Tris.HCl, pH 7.6, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) containing protease inhibitor cocktail (Invitrogen), centrifuged at 12,000 X g for 10 min, and the supernatant was assayed for total protein [3,4]. Equal amounts of protein (50-75μg) were loaded onto 10-12% SDS-PAGE (Bio-Rad Laboratories, Hercules, CA). The proteins were electrophoretically transferred onto methanol activated Immuno-Blot PVDF membranes, which were probed with specific Abs that recognize StAR, aromatase, and β-actin. Following overnight incubation with primary Abs, the membranes were washed and incubated with appropriate horseradish peroxidase-conjugated secondary Abs against rabbit or mouse IgG for 1h at RT. The immunodetection of different proteins was determined using a Chemiluminescence kit, exposed to X-ray films (Phenix Research, Candler, NC), and the intensity of bands was quantified using a computer-assisted image analyzer (Quantity One Software, Bio-Rad Laboratories), as described previously [3,4].

2.3. Enzyme-Linked Immunosorbent Assay (ELISA)

17β-estradiol (E2) levels in cell culture media were determined using ELISA Kit from Cayman Chemical (Ann Arbor, MI), according to manufacturer's protocol [17], optimized further for better efficacy. Briefly, culture media collected from various cell lines and/or treatment groups were extracted with diethyl ether (5:1, v/v), snap froze samples in ice/ethanol bath, and poured top solvent layer into another tube. Solvent samples containing E2

were dried with air or in a speedvac, resuspended in assay buffer, and E2 levels were measured. The sensitivity of E2 assay was 15pg/ml, and intra-assay percentage coefficient of variation was below 10%. Assays were performed at duplicates and absorbance was read at 412nm using an Infinite M100 PRO Microplate Reader (Tecan, Mannedorf, Switzerland).

2.4. Immunoprecipitation

Cells (MCF7 and H295R) were seeded at 1×10^6 per 100-mm cell culture dishes. Following 24h of plating, cells were treated without (DMSO) or with HDAC inhibitors (either single or increasing doses) for varying time periods, as specified in different experiments. Cells were then harvested and homogenized in a lysis buffer (50mM Tris.HCl, pH 7.4, 150mM NaCl, 1% Triton X-100, 0.5% NP-40, 10% glycerol, containing protease inhibitor cocktail (Invitrogen), 1μM Trichostatin A and 1mM nicotinamide), as described previously [17]. Total protein (1.2-1.5mg) was immunoprecipitated with 1μg of mouse IgG or acetyl lysine (Ac-Lys) Ab in a total volume of 1ml lysis buffer, for 16h at 4°C on a Nutator (Fisher Scientific, Waltham, MA). Protein-antibody-complexes were incubated with Protein G Dynabeads (Invitrogen) for 2h at 4°C. Immune complexes were washed for 4-6 times with lysis buffer, and samples were processed and analyzed by SDS-PAGE (BioRad).

2.5. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Cells (MCF7 and H295R) were seeded at 5×10^6 per dish in 150-mm cell culture dishes. After 24h of plating, cells were washed with 0.01M PBS and treated without (DMSO) or with Panobinostat (10nM), SAHA (1μM), inhibitor IV (1μM), inhibitor VII (1μM) for 45min. After treatments, cells were washed, collected, and homogenized in immunoprecipitation lysis buffer at 4°C. Total protein (13-16mg) was incubated with 3-4μg anti-StAR Ab for 16h at 4°C. After incubation, Protein G Dynabeads (Invitrogen) were added to protein-antibody-complexes and incubated for an additional 2h at 4°C. Immunoprecipitates were washed for 6 times with lysis buffer and final pellets were sent to Applied Biomics Inc (Hayward, CA) for the identification of StAR acetyl lysine site(s) by LC-MS/MS (Thermo Fisher Ultimate 3000, Milford, MA) [17].

2.6. Statistical analysis

All experiments were repeated at least three times. Data were analyzed either by student *t*-test or analysis of variance followed by Fisher's protected least significant difference test using Statview (Abacus Concepts, Inc., Berkeley, CA). Data presented are the mean ±SE, and $p<0.05$ was considered statistically significant.

3. Results

3.1. Relative expression of StAR and aromatase proteins and E2 synthesis in non-cancerous and cancerous breast cell lines

The hypothesis that hormone sensitive breast cancer involves a gain of function of the StAR protein in the transport of cholesterol, resulting in ample precursor availability for E2 synthesis in promoting tumorigenesis, was examined. As illustrated in Fig. 1A, expression of the StAR protein was markedly higher in all three ER+ MCF7, MBA-MD-361, and T-47D cell lines, over the responses seen in non-cancer mammary epithelial cell lines (MCF10A

and MCF12F). StAR protein expression was moderate ($p<0.05$) in three different TNBC cell lines (MBA-MD-231, MBA-MD-468, and BT-549). Expression of aromatase, which converts androgens to estrogens, was similar in non-cancer and breast cancer cell lines evaluated (Fig. 1A), consistent with a recent report from our laboratory [15]. Accumulation of E2 in media qualitatively followed expression of StAR protein, and were 14 ± 5.6 and 3 ± 1.8 fold in ER+ and TNBC respectively, when compared with values obtained in normal mammary epithelial cells (Fig. 1B). These results demonstrate a close correlation between StAR protein expression and E2 synthesis in various non-cancer and breast cancer cell line models.

3.2. Identification of StAR protein acetylation and its correlation to HDAC inhibition in MCF7 cells, and determination of StAR acetyl lysine residues by LC-MS/MS

Almost all proteins in eukaryotic cells are altered by various post-translational modifications (PTMs). We recently reported acetylation of aromatase in pertinent cancer cells [17]. To determine if StAR protein is acetylated in hormone sensitive breast cancer, immunoprecipitation studies were performed using MCF7 cell line. The data presented in Figs. 2A&C revealed acetylation of endogenous StAR in MCF7 cells. Treatment of these cells with an FDA approved HDAC inhibitor (HDACi), panobinostat, at a clinical dose (10nM), for 0-180min, caused increases in StAR protein acetylation (Ac-StAR) in a temporal response manner. Induction of Ac-StAR was evident ($p<0.05$) at 20min, optimal between 45 and 60min, slightly decreased thereafter but remained elevated over basal at 180min, the maximum time point tested. Under similar experimental conditions, expression of total StAR (T-StAR) was unaltered up to 90min, began to diminish thereafter, and decreased ($p<0.05$) at 180min (Figs. 2B&C). E2 levels in media at various time points followed patterns as those observed with T-StAR expression (Figs. 2B&C).

Acetylation of the StAR protein and its correlation to HDAC inhibition was further assessed with a number of HDAC inhibitors affecting various HDACs. MCF7 cells treated with SAHA (1μM), panobinostat (PANO, 10nM), inhibitor IV (IV, 1μM), inhibitor VII (VII, 1μM), entinostat (1μM), practinostat (PRAC, 1μM), and romidepsin (ROMI, 100nM), for 45min at preclinical and clinical doses, demonstrated increases ($p<0.01$) in Ac-StAR between 2.8 and 3.4 fold in response to SAHA, PANO, IV and VII and ROMI (Figs. 2D&F). Expression of T-StAR was unaltered in response to any of these HDAC inhibitors (Fig. 2E).

To obtain more insight into this PTM, acetyl lysine residue(s) in the StAR protein was identified, under basal and HDACi treated conditions, using LC-MS/MS. MCF7 cells treated without (DMSO) or with SAHA (1μM), panobinostat (10nM), IV (1μM) and VII (1μM) recognized a number of StAR acetyl lysine residues (Supplemental Table 1). Specifically, three StAR acetyl lysine residues at positions 111, 238, and 253 were basally identified in MCF7 cells. Treatment with HDAC inhibitors was associated with four additional residues at K21, K152, K162, and K248. Locations of each of these StAR acetyl lysine residues and their interacting domains are illustrated in Supplemental Table 1.

3.3. Acetylation of the StAR protein and its correlation to HDAC inhibition and E2 synthesis in adrenocortical H295R cells

To further verify StAR acetylation, immunoprecipitation studies were performed using the H295R cell line (that closely resembles its normal counterpart and has been widely used in studying adrenal physiological functions). H295R cells treated without (DMSO) or with PANO (10nM), SAHA (1μM), IV (1μM), and entinostat (1μM), for 45min, induced ($p<0.05$) Ac-StAR in response to PANO, SAHA and IV, over untreated cells (Fig. 3A). Entinostat displayed no apparent effect on induction of Ac-StAR. T-StAR levels were unchanged in response to any of these HDAC inhibitors (Fig. 3A). StAR protein was also found to be acetylated endogenously in H295R cells, in which LC-MS/MS analysis identified four acetylated residues at K98, K107, K111, and K118 in the StAR protein (Supplemental Table 1). Four additional residues at K7, K213, K236, and K238 were identified in response to PANO, SAHA and IV.

In order to understand the inhibition of HDACs on StAR mediated E2 synthesis, H295R cells were treated with a number of HDAC inhibitors, for 24h, at two different concentrations (Fig. 3B). The results show that SAHA and PANO at both doses decreased T-StAR expression and E2 synthesis, when compared with untreated cells, indicating that HDAC inhibition targets StAR and, thus, steroid biosynthesis. Whereas IV affected ($p<0.05$) both StAR and E2 levels at 1μM, the doses utilized for entinostat were ineffective in repressing the steroidogenic response.

3.4. Impact of HDAC inhibition on acetylation and expression of StAR and their correlation to E2 accumulation in MCF7 cells

To further determine the efficacy of HDAC inhibition on StAR expression and E2 synthesis, MCF7 cells were treated without or with panobinostat and IV, at increasing concentrations (0-2μM) for either 45min (Figs. 4A&C) or 24h (Figs. 4B&D). The results demonstrate that both panobinostat and IV resulted in increases in Ac-StAR, but not T-StAR, in concentration dependent manners. Ac-StAR was induced ($p<0.01$) in response to 10nM panobinostat, and displayed no additional effects with higher concentrations (Fig. 4A). Induction of Ac-StAR by IV was evident with 100nM ($p<0.05$), increased thereafter, reaching a plateau between 1 and 2μM, when compared with untreated cells (Fig. 4C).

In additional studies, the effects of panobinostat and IV were examined on T-StAR expression and E2 synthesis. As illustrated in Figs. 4B&D, MCF7 cells treated with panobinostat and IV (0-2μM), individually, for 24h, decreased both T-StAR and E2 levels in a concentration dependent manner. Panobinostat and IV were capable of suppressing E2 synthesis maximally by ~78% and ~66%, when compared their levels with respective controls. These results demonstrate that inhibition of HDACs effectively inhibited T-StAR expression that mirrored E2 synthesis in hormone sensitive MCF7 cells.

4. Discussion

Regulation of the steroidogenic machinery is instrumental to proper functioning of a variety of biological activities. Since StAR regulates steroid biosynthesis, its expression must be

finely regulated such that it is available at appropriate times and responds to various signals. Malfunction in androgen and/or estrogen biosynthesis has been implicated in the development and growth of a variety of hormone responsive cancers [10,18]. It is unequivocal that the elevated level of E2 has been linked to the pathogenesis of ER+ breast cancer. Since treatment of hormone sensitive breast cancer with aromatase inhibitors often leads to resistance, it is important to identify other potential therapeutic targets. As such, considerable attention has been placed upon the utilization of HDAC inhibitors in the management of a variety of malignancies, including breast cancer [7,13,19]. The contribution of StAR was explored to determine its potential involvement in the development of breast cancer. The present studies extend previous observations and expand our understanding by elucidating molecular events in which StAR protein is not only abundantly expressed, but also it is acetylated in ER+ breast cancer cells, and inhibition of HDACs decreases StAR expression and E2 synthesis, suggesting therapeutic relevance of this cholesterol transporter in hormone sensitive breast cancer.

Our current results demonstrate that StAR protein was almost undetectable in normal mammary epithelial cells; however, its expression was markedly higher in ER+ breast cancer cell line models. Alternatively, TNBC cells displayed moderate expression of StAR. Accumulation of E2 in media was closely correlated with StAR protein expression in both non-cancerous and cancerous breast cell lines. Aberrant expression of StAR, along with the increased levels of E2, designates that StAR acts as an oncogene in hormone sensitive breast cancer cells. It is conceivable that abundant expression of StAR facilitates unusual cholesterol delivery to the inner mitochondrial membrane and, as a consequence, additional precursor for E2 in promoting breast tumorigenesis. In this connection, it is worth noting that StAR related lipid transfer protein 3 (also known as metastatic lymph node 64), a late endosomal membrane protein with structural and functional homology with StAR, has been shown to be overexpressed in HER2+ breast cancer [20, 21]. It is well-known that estrogen levels in the majority of hormone dependent breast cancers are strikingly higher than those found in either circulation or non-cancerous counterpart [5,9,22]. A central question concerns profound expression of the StAR protein in ER+ breast cancer. Previously, we demonstrated that cAMP-mediated mechanisms phosphorylate StAR and this PTM enhances the cholesterol transporting capacity of StAR to the mitochondria for optimal steroid biosynthesis [3,23]. Mitochondria play crucial roles in many physiological and/or pathological processes, and improvements in proteomic technologies have frequently identified lysine acetylation sites in mitochondrial proteins that exhibit both positive and negative effects on protein function [24,25]. In the present study, mitochondrially localized StAR was identified as a novel acetylated protein, in which a total of eleven acetyl lysine residues were recognized under basal and HDACi treated conditions, surmising these lysine residues differently influences the steroidogenic response. It is tempting to speculate that while the identified acetyl lysine residues on endogenous StAR are connected with higher E2 levels, HDACi treated ones associate with decreased E2 synthesis. However, StAR acetylation and its correlation to steroid biosynthesis may be context specific and involve discrete mechanisms in classical and non-classical target tissues. Studies designed to elucidate the functional relevance of each of these StAR acetyl lysine residues identified, either endogenously or in response to HDAC inhibitors, should lead to a better

understanding of the involvement of this previously uncharacterized PTM on StAR expression and steroid biosynthesis, and are currently underway.

There is increasing evidence that enhanced expression and/or activity of aromatase is one of the key events for elevated intra-tumoral production of estrogen in malignant breast tissues [5,7,26]. Nevertheless, aromatase has been a molecular target for therapeutic approaches for a number of estrogen dependent cancers, including breast cancer. Noteworthy, however, E2 levels did not correlate with aromatase protein expression, but to StAR, in three different breast cell lines, reinforcing the notion that StAR plays an indispensable role in the regulation of steroid biosynthesis [2]. In line with higher expression of the StAR protein in breast cancer *in vitro*, analyses of genomic and molecular profiles of key steroidogenic factors in human primary breast cancer tumors, available in the TCGA database, indicated that amplification of the *StAR* gene correlates with breast cancer mortality (Manna PR et al, unpublished observation).

An intriguing aspect of the present studies is the HDAC inhibition mediated repression of StAR expression and E2 synthesis in MCF7 cells. The coordinate association of the StAR protein in breast cancer was exemplified by three different scenarios: i) ER+ breast cancer cells displayed markedly higher expression of the StAR protein, concomitant with E2 synthesis, in comparison to their normal counterpart, ii) novel StAR acetylation patterns are induced with inhibition of HDACs, and iii) HDAC inhibitors that affected StAR protein expression also decreased E2 synthesis. The inhibition of HDACs has been shown to have multiple targets in cancer cells. We reported previously that inhibition of SIRT1/2 (class III HDACs) influences acetylation and activity of aromatase in breast cancer cells [17,27]. It has been shown that HDAC inhibitors promote the degradation of Dishevelled proteins [27], decreases cell migration and Rac activation [28], and triggers ubiquitin dependent proteosomal degradation of DNA methyltransferase 1 in breast cancer cells [29]. HDAC I inhibitor romidepsin induces acetylation of histone 3 and apoptosis, and subsequently suppresses vascular epithelial growth factor and hypoxia-inducible factor 1α in breast cancer cells [30]. Panobinostat, a pan-deacetylase inhibitor, is capable of decreasing aromatase, either alone or in combination with an aromatase inhibitor letrozole, in MCF7/human adrenocortical H295R cells [31]. Both SAHA and panobinostat have been shown to acetylate Hsp90 and degrades ER, ErbB2 and HDAC6 in TNBC cells [32]. Thus, HDAC inhibitors have been demonstrated to produce a variety of effects, including cell cycle arrest, apoptosis, differentiation and anti-angiogenesis, in tumor/cancer cells [13,33]. Our present data added information to the growing list of HDACi mediated effects documenting that inhibition of HDACs targets cholesterol transporter StAR and, thus, E2 synthesis, in hormone sensitive breast cancer.

Taken together, aberrant high expression of the StAR protein, with elevated levels of E2, is a plausible mechanism in the development and progression of ER+ breast cancer affecting survival. It is highly likely that StAR delivers abnormal cholesterol to the mitochondria resulting in an adequate availability of androgen precursors and thus E2 in promoting breast tumorigenesis. Hence, StAR may be considered to have prognostic value in breast cancer. A number of HDAC inhibitors that repressed StAR expression also decreased E2 synthesis in

MCF7 cells, underlining that StAR could be targeted therapeutically in the prevention and/or treatment of hormone sensitive breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported in part by National Institute of Health grant CA155223 and Cancer Prevention Research Institute of Texas (CPRIT) Award RR140008 to KP.

References

[1]. Miller WL, Bose HS, Early steps in steroidogenesis: intracellular cholesterol trafficking, J Lipid Res, 52 (2011) 2111–2135. [PubMed: 21976778]

[2]. Manna PR, Stetson CL, Slominski AT, Pruitt K, Role of the steroidogenic acute regulatory protein in health and disease, Endocrine, 51 (2016) 7–21. [PubMed: 26271515]

[3]. Manna PR, Cohen-Tannoudji J, Counis R, Garner CW, Huhtaniemi I, Kraemer FB, Stocco DM, Mechanisms of action of hormone-sensitive lipase in mouse Leydig cells: its role in the regulation of the steroidogenic acute regulatory protein, J Biol Chem, 288 (2013)8505–8518. [PubMed: 23362264]

[4]. Manna PR, Sennoune SR, Martinez-Zaguilan R, Slominski AT, Pruitt K, Regulation of retinoid mediated cholesterol efflux involves liver X receptor activation in mouse macrophages, Biochem Biophys Res Commun, 464 (2015) 312–317. [PubMed: 26119689]

[5]. Bulun SE, Lin Z, Zhao H, Lu M, Amin S, Reierstad S, Chen D, Regulation of aromatase expression in breast cancer tissue, Ann N Y Acad Sci, 1155 (2009) 121–131. [PubMed: 19250199]

[6]. Folkerd E, Dowsett M, Sex hormones and breast cancer risk and prognosis, Breast, 22 Suppl 2 (2013) S38–43. [PubMed: 24074790]

[7]. Manna PR, Molehin D, Ahmed AU, Dysregulation of Aromatase in Breast, Endometrial, and Ovarian Cancers: An Overview of Therapeutic Strategies, Prog Mol Biol Transl Sci, 144 (2016) 487–537. [PubMed: 27865465]

[8]. Dai X, Xiang L, Li T, Bai Z, Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes, J Cancer, 7 (2016) 1281–1294. [PubMed: 27390604]

[9]. Zhao H, Zhou L, Shangguan AJ, Bulun SE, Aromatase expression and regulation in breast and endometrial cancer, J Mol Endocrinol, (2016) R19–33. [PubMed: 27067638]

[10]. Chumsri S, Howes T, Bao T, Sabnis G, Brodie A, Aromatase, aromatase inhibitors, and breast cancer, J Steroid Biochem Mol Biol, 125 (2011) 13–22. [PubMed: 21335088]

[11]. Palmieri C, Patten DK, Januszewski A, Zucchini G, Howell SJ, Breast cancer: current and future endocrine therapies, Mol Cell Endocrinol, 382 (2014) 695–723. [PubMed: 23933149]

[12]. Johnston SR, Enhancing Endocrine Therapy for Hormone Receptor-Positive Advanced Breast Cancer: Cotargeting Signaling Pathways, J Natl Cancer Inst, 107 (2015) doi: 10.1093/jnci/djv212.

[13]. West AC, Johnstone RW, New and emerging HDAC inhibitors for cancer treatment, J Clin Invest, 124 (2014) 30–39. [PubMed: 24382387]

[14]. Pruitt K, Molecular and Cellular Changes During Cancer Progression Resulting From Genetic and Epigenetic Alterations, Prog Mol Biol Transl Sci, 144 (2016) 3–47. [PubMed: 27865461]

[15]. Castro-Piedras I, Sharma M, den Bakker M, Molehin D, Martinez EG, Vartak D, Pruitt WM, Deitrick J, Almodovar S, Pruitt K, DVL1 and DVL3 differentially localize to CYP19A1 promoters and regulate aromatase mRNA in breast cancer cells, Oncotarget, 9 (2018) 35639–35654. [PubMed: 30479694]

[16]. Manna PR, Stetson CL, Daugherty C, Shimizu I, Syapin PJ, Garrel G, Cohen-Tannoudji J, Huhtaniemi I, Slominski AT, Pruitt K, Stocco DM, Up-regulation of steroid biosynthesis by retinoid signaling: Implications for aging, Mech Ageing Dev, 150 (2015) 74–82. [PubMed: 26303142]

[17]. Molehin D, Castro-Piedras I, Sharma M, Sennoune SR, Arena D, Manna PR, Pruitt K, Aromatase Acetylation Patterns and Altered Activity in Response to Sirtuin Inhibition, Mol Cancer Res, 16 (2018) 1530–1542. [PubMed: 29921733]

[18]. Capper CP, Rae JM, Auchus RJ, The Metabolism, Analysis, and Targeting of Steroid Hormones in Breast and Prostate Cancer, Horm Cancer, 7 (2016) 149–164. [PubMed: 26969590]

[19]. Li Y, Seto E, HDACs and HDAC Inhibitors in Cancer Development and Therapy, Cold Spring Harb Perspect Med, 6 (2016) doi: 10.1101/cshperspect.a026831.

[20]. Alpy F, Boulay A, Moog-Lutz C, Andarawewa KL, Degot S, Stoll I, Rio MC, Tomasetto C, Metastatic lymph node 64 (MLN64), a gene overexpressed in breast cancers, is regulated by Sp/KLF transcription factors, Oncogene, 22 (2003) 3770–3780. [PubMed: 12802284]

[21]. Vassilev B, Sihto H, Li S, Holtta-Vuori M, Ilola J, Lundin J, Isola J, Kellokumpu-Lehtinen PL, Joensuu H, Ikonen E, Elevated levels of StAR-related lipid transfer protein 3 alter cholesterol balance and adhesiveness of breast cancer cells: potential mechanisms contributing to progression of HER2-positive breast cancers, Am J Pathol, 185 (2015) 987–1000. [PubMed: 25681734]

[22]. Simpson E, Santen RJ, Celebrating 75 years of oestradiol, J Mol Endocrinol, 55 (2015) T1–20. [PubMed: 26438567]

[23]. Manna PR, Chandrala SP, King SR, Jo Y, Counis R, Huhtaniemi IT, Stocco DM, Molecular mechanisms of insulin-like growth factor-I mediated regulation of the steroidogenic acute regulatory protein in mouse leydig cells, Mol Endocrinol, 20 (2006) 362–378. [PubMed: 16166197]

[24]. Verdin E, Ott M, 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond, Nat Rev Mol Cell Biol, 16 (2015) 258–264. [PubMed: 25549891]

[25]. Baeza J, Smallegan MJ, Denu JM, Mechanisms and Dynamics of Protein Acetylation in Mitochondria, Trends Biochem Sci, 41 (2016) 231–244. [PubMed: 26822488]

[26]. Simpson ER, Misso M, Hewitt KN, Hill RA, Boon WC, Jones ME, Kovacic A, Zhou J, Clyne CD, Estrogen--the good, the bad, and the unexpected, Endocr Rev, 26 (2005) 322–330. [PubMed: 15817672]

[27]. Holloway KR, Barbieri A, Malyarchuk S, Saxena M, Nedeljkovic-Kurepa A, Cameron Mehl M, Wang A, Gu X, Pruitt K, SIRT1 positively regulates breast cancer associated human aromatase (CYP19A1) expression, Mol Endocrinol, 27 (2013) 480–490. [PubMed: 23340254]

[28]. Saxena M, Dykes SS, Malyarchuk S, Wang AE, Cardelli JA, Pruitt K, The sirtuins promote Dishevelled-1 scaffolding of TIAM1, Rac activation and cell migration, Oncogene, 34 (2015) 188–198. [PubMed: 24362520]

[29]. Zhou Q, Agoston AT, Atadja P, Nelson WG, Davidson NE, Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells, Mol Cancer Res, 6 (2008) 873–883. [PubMed: 18505931]

[30]. Robertson FM, Chu K, Boley KM, Ye Z, Liu H, Wright MC, Moraes R, Zhang X, Green TL, Barsky SH, Heise C, Cristofanilli M, The class I HDAC inhibitor Romidepsin targets inflammatory breast cancer tumor emboli and synergizes with paclitaxel to inhibit metastasis, J Exp Ther Oncol, 10 (2013) 219–233. [PubMed: 24416998]

[31]. Chen S, Ye J, Kijima I, Evans D, The HDAC inhibitor LBH589 (panobinostat) is an inhibitory modulator of aromatase gene expression, Proc Natl Acad Sci U S A, 107 (2010) 11032–11037. [PubMed: 20534486]

[32]. Edwards A, Li J, Atadja P, Bhalla K, Haura EB, Effect of the histone deacetylase inhibitor LBH589 against epidermal growth factor receptor-dependent human lung cancer cells, Mol Cancer Ther, 6 (2007) 2515–2524. [PubMed: 17876048]

[33]. Xu WS, Parmigiani RB, Marks PA, Histone deacetylase inhibitors: molecular mechanisms of action, Oncogene, 26 (2007) 5541–5552. [PubMed: 17694093]

Highlights:

- The StAR protein is abundantly expressed in breast cancer but not in normal mammary epithelial cells.
- StAR is identified as a novel acetylated protein.
- Inhibition of HDACs decreases StAR and estrogen levels in MCF7 cells.
- StAR can be considered as a novel therapeutic target in managing breast cancer.

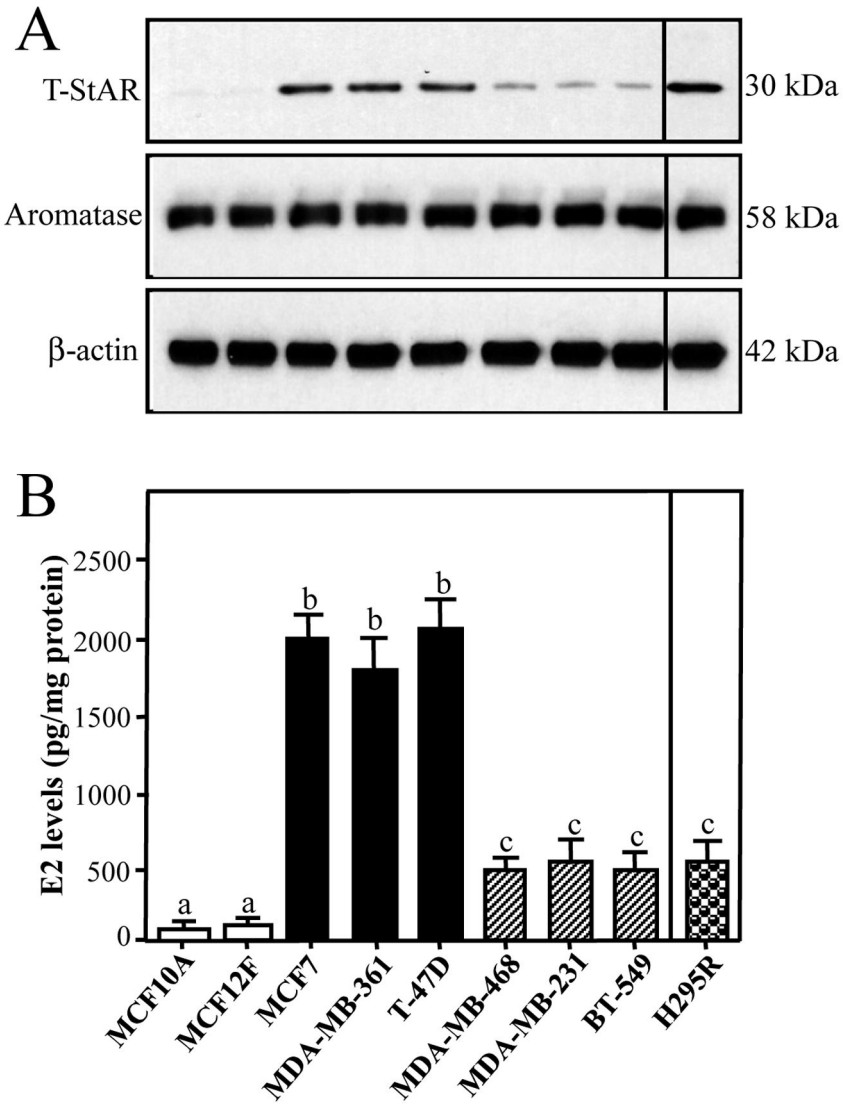

Fig. 1.

Relative expression of StAR and aromatase proteins, and E2 synthesis, in human normal mammary epithelial (MCF10A and MCF12F), ER+ breast cancer (MCF7, MBA-MD-361, and T-47D), TNBC (MBA-MD-468, MBA-MD-231, and BT-549), and H295R, cell lines. These cells were cultured with appropriate media, and were harvested in RIPA buffer at 75-80% confluence. Cells and media were then processed for whole cell extract preparation and E2 extraction by diethyl ether, respectively. Representative immunoblots illustrate total StAR (T-StAR) and aromatase protein levels in different cell lines (A). Immunoblots shown are representative of 4 independent experiments. β-actin expression was assessed as a loading control. B, E2 levels in media from different cell lines were determined by ELISA and presented as pg/mg protein (n=4, ±SE). Different letters above the bars indicate that these groups differ significantly from each other at least at $p<0.05$.

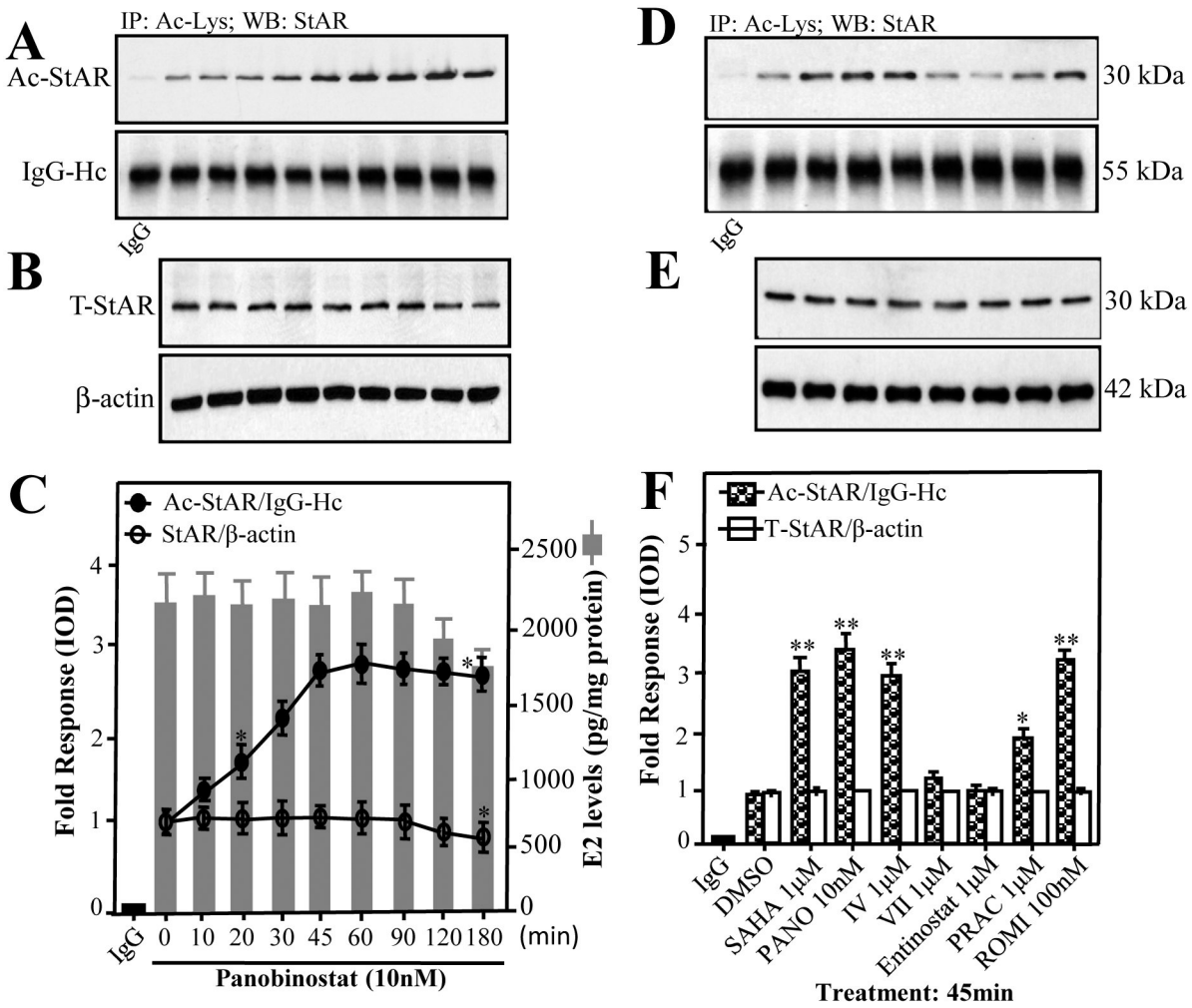

Fig. 2.

Acetylation and expression of StAR in response to HDAC inhibitors, and their correlation to E2 synthesis in MCF7 cells. Cells (1×10^6 per dish) were plated in 100-mm dishes 24h before treatments. Cells were then treated without or with panobinostat (10nM) for 0-180min (A-C) or other HDAC inhibitors for 45min (D-F). Following treatments, cells were collected, extracted with lysis buffer, and processed for either immunoprecipitation or immunoblotting studies, as described in *Materials and methods*. Representative immunoblots illustrate acetylation (Ac-StAR) and expression of StAR (T-StAR) in different treatment groups. IgG heavy chain (IgG-Hc) and β-actin expression were assessed for loading controls in immunoprecipitation and immunoblotting, respectively. Integrated optical density (IOD) values of Ac-StAR and T-StAR in each band were quantified and normalized with corresponding IgG-Hc and β-actin expression, and presented as fold response (C,F). E2 levels in media at each time point were determined by ELISA and presented as pg/mg protein (C). Data are representative of 3-4 independent experiments. *, $p<0.05$; **, $p<0.01$ vs. basal.

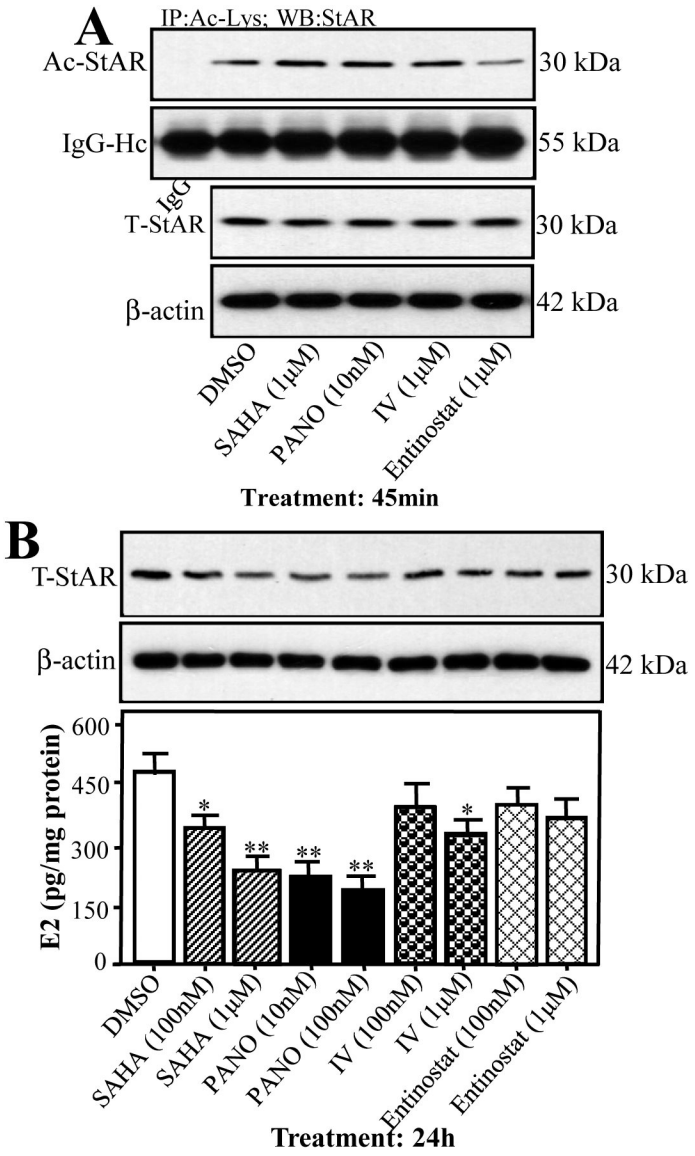

Fig. 3.

Effects of a variety of HDAC inhibitors on acetylation and expression of StAR and E2 synthesis in H295R cells. Cells (5×10^5 per 6-well dish or 1×10^6 per 100-mm dish) were plated 24h before treatments. Cells were then treated without or with SAHA (100nM and/or 1μM), PANO (10nM and/or 100nM), IV (100nM and/or 1μM) and entinostat (100nM and/or 1μM), for either 45min (A) or 24h (B). Following treatments, cells were processed for both immunoprecipitation and immunoblotting studies as indicated in the legend of Fig. 2. Representative immunoblots illustrate Ac-StAR and T-StAR in different panels. IgG-Hc and β-actin expression were assessed for loading controls in immunoprecipitation and immunoblotting, respectively. E2 levels in media from different treatment groups were determined by ELISA and presented as pg/mg protein (B). Data are representative of 3 independent experiments. *, $p<0.05$; **, $p<0.01$ vs. basal.

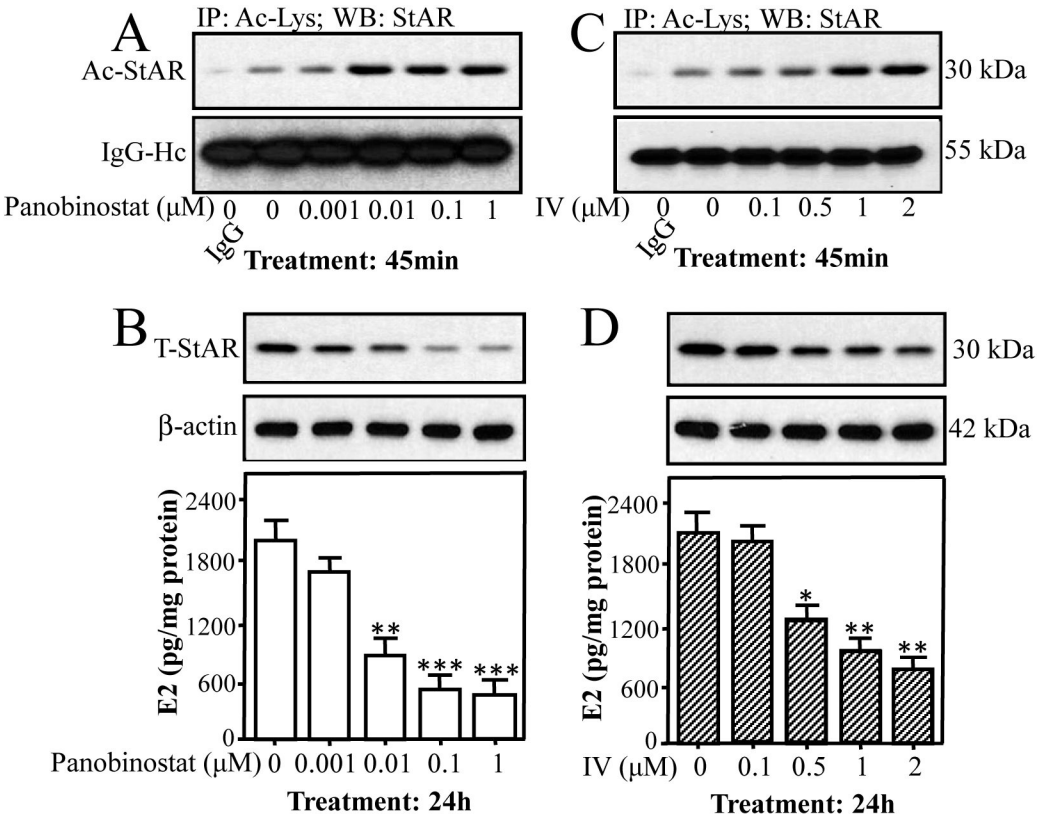

Fig. 4.

Inhibition of HDACs on acetylation and expression of StAR and E2 synthesis in MCF7 cells. Cells were plated either at 1×10^6 per 100-mm dish or 5×10^5 per 6-well dish for immunoprecipitation and immunoblotting, respectively. Cells were treated without or with increasing doses (0-2μM) of either panobinostat or IV, for either 45min (A&C) or 24h (B&D), and subjected to immunoprecipitation and immunoblotting analyses, as described in the legend of Fig. 2. Representative immunoblots (n=3-4) illustrate Ac-StAR and T-StAR in response to either panobinostat (A&B) or IV (B&D). IgG-Hc and β-actin expression were assessed for loading controls in immunoprecipitation and immunoblotting, respectively. B&D; E2 levels in media were determined by ELISA and presented as pg/mg protein (n=3, ±SE). *, $p<0.05$; **, $p<0.01$; ***, $p<0.0001$ vs. basal.