Molecular and Cellular Endocrinology 476 (2018) 185–197

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Increase in hyaluronic acid degradation decreases the expression of estrogen receptor alpha in MCF7 breast cancer cell line

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ARTICLE INFO

Keywords:
Estrogen signaling
Hyaluronic acid
Hyaluronidase
Breast cancer
Hormonal therapy resistance

ABSTRACT

The loss of estrogen receptor α (ERα) expression in breast cancer constitutes a major hallmark of tumor progression to metastasis and is generally correlated to a strong increase in Hyaluronic Acid (HA) turnover. The aim of our study was to search for a putative link between these two major events of breast cancer progression in the estrogen receptor-positive (ER+) MCF7 breast cancer cell line. The increase in HA turnover was performed by stable overexpression of the standard CD44 (CD44S) isoform and also by treatment with exogenous Hyaluronidase (Hyal) to allow an increase in HA catabolism. Stable overexpression of CD44S in MCF7 cells was correlated to a decrease in ESR1 gene expression, which did not lead to alteration of estrogen response. Moreover, our results showed that the exposure to exogenous Hyal stimulates the proliferation and strongly decreases the expression of ERα whatever the expression level of CD44 in the MCF7 cell line. The culture in the presence of Hyal led to the decrease in estrogens responsiveness and to hormonal therapy resistance. The effect on growth is correlated to the activation of MAPK/ERK and PI3K/Akt signaling pathways while the Hyal-induced down-regulation of ESR1 gene expression involves the activation of PI3K/Akt and NF-κB signaling pathways. Many of our data suggest that the effects of Hyal described here could be related to the activation of TLR signaling. Taken together, our results demonstrate that the increase in HA degradation could be involved in breast cancer progression and in resistance to hormonal therapy.

1. Introduction

Estrogens signaling pathway plays a major role in the initiation and development of breast cancers. Indeed, approximately 70% of breast cancers are ERα positive and hormone dependent (Osborne et al., 2001). Thereby, the use of hormonal therapy targeting the estrogen receptor signaling pathways is the most effective treatment for this pathology and ERα expression thus serves as a prognostic marker of responsiveness to the treatment (Williams and Lin, 2013). Post-surgery endocrine therapy, such as inhibition of estrogens synthesis using aromatase inhibitors or inhibition of estrogens signaling pathway using selective estrogen receptor modulator (SERM) or selective estrogen receptor down-regulator (SERD), led to a significant improvement of survival and a reduction in disease relapse (Lumachi et al., 2013). However, effectiveness of endocrine therapy is often limited by the occurrence of resistance to the treatment whose mechanisms involve the loss of ERα expression and/or the activation of hormone-independent mitogenic signaling (Osborne and Schiff, 2011).

The progression of breast cancers to metastasis is initiated by the epithelial-mesenchymal transition process (EMT) (Creighton et al, 2010a). Although it is strongly involved in breast cancer development and progression, the estrogen signaling pathway exerts a protective role against the progression to metastasis (Guttilla et al., 2012). Indeed, the activation of ERα signaling allows the maintaining of an epithelial phenotype by repressing EMT-promoting transcription factors in breast cancer (Ye et al., 2008, 2010; Bouris et al., 2015). Moreover, the silencing of ERα expression in ER + breast cancer lines promotes cell migration and invasion (Ye et al., 2010; Bouris et al., 2015). Lastly,

Abbreviations: CD44, cluster of differentiation 44; CV, crystal violet; E2, 17β-estradiol; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; GPI, glycosylphosphatidylinositol; HA, hyaluronic acid; HAS, hyaluronan synthase; HMW HA, high molecular weight hyaluronic acid; Hyal, hyaluronidase; LMW HA, low molecular weight hyaluronic acid; LPS, lipopolysaccharides; NFκB, nuclear factor κ B; oHA, oligosaccharides of hyaluronic acid; PR, progesterone receptor; SERD, selective estrogen receptor down-regulator; SERM, selective estrogen receptor modulator; TFF1, trefoil factor 1; TGFβ, transforming growth factor β; TLR, toll-like receptor

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https://doi.org/10.1016/j.mce.2018.05.008
Received 18 December 2017; Received in revised form 4 May 2018; Accepted 8 May 2018

ERα-mediated signaling has been described to antagonize signaling pathways that lead to EMT such as TGFβ signaling (Goto et al., 2011) and NFκB signaling (Wang et al., 2009). The loss of ERα expression therefore constitutes a major step in the initiation of breast cancer metastasis (Guttilla et al., 2012).

The tumor microenvironment plays a crucial role in breast cancer progression to metastasis. Indeed, the infiltration of tumors by inflammatory cells contributes to the increase in malignancy. Moreover, an extensive remodeling of the extracellular matrix (ECM) surrounding solid tumors is closely related with their development and progression (Iijima et al., 2011). Hyaluronic acid (HA), a major component of ECM, is known to be involved in breast cancer progression to metastasis (Toole, 2004). HA is a high molecular weight polysaccharide composed of repeated disaccharides of glucuronic acid and N-acetylglucosamine. The level of extracellular HA is subjected to a dynamic turnover involving a sophisticated balance between its biosynthesis and its catabolism. HA synthesis is ensured by an enzyme family named hyaluronan synthases and composed of three related isoforms: HAS1, HAS2 and HAS3 and its catabolism is regulated by an enzymatic degradation involving an enzyme family called hyaluronidases (Hyal) (Stern, 2004). Six different genes encoding for Hyal have been described, HYAL1, HYAL2, HYAL3, HYAL4, HYALP1 and PH-20, but the major Hyal involved in HA degradation in somatic cells are HYAL1 and HYAL2 (Stern, 2004). The degradation of HA by the cells involves the action of CD44, which is the main HA receptor. Indeed, CD44 in association with GPI-anchored HYAL2 promotes the tethering of high molecular weight (HMW) HA to the membrane. The HA fragments produced by HYAL2 are internalized and then degraded in small disaccharides by HYAL1 in the lysosomes (Stern, 2004). In addition to its role in adhesion and HA degradation and while devoid of intrinsic kinase activity, CD44 can trigger the activation of intracellular signaling pathway involved in the regulation of growth and motility (Louderbough and Schroeder, 2011).

Overall, the progression of many types of cancers is associated with an increase in expression of HAS, HYAL and also CD44 and, in some cases, HA level serves as a prognostic marker for tumor progression (Toole, 2004). Thus, an overexpression of HAS2, HYAL2 and CD44 has been reported in highly invasive breast cancer cell lines (Udabage et al., 2005a). Moreover, the silencing of HAS2 gene expression in the invasive MDA-MD-231 breast cancer cell line induced a decrease in expression of HYAL2 and CD44 and also led to the inhibition of tumorigenesis and to the progression of breast cancer (Udabage et al., 2005b). The effect of HA in the acquisition of aggressive characteristics is closely related to its size. Indeed, it is well established that HA fragments resulting from HMW HA catabolism strongly promote the metastasis of different types of cancers (Iijima et al., 2011; Stern et al., 2006). In breast cancer, a significant correlation between high levels of low molecular weight (LMW) HA quantified in sera and lymph node metastasis has been reported and the inhibition of LMW HA production led to a significant decrease in migration and invasion (Wu et al., 2014). The action mechanism of HA fragments in metastasis is related to their abilities to induce inflammatory responses in the tumoral microenvironment (Nikitovic et al., 2015). This effect of HA degradation products involves the activation of innate immune responses by interaction with TLR receptors and to a lesser extent with CD44 (Nikitovic et al., 2015).

According to the literature analysis, the progression of breast cancer is characterized by the loss of estrogen dependency associated with an increase in HA turnover. This suggests a direct relationship between these two major events of breast cancers metastasis. Recently, a study carried out in our laboratory demonstrated an inhibition of estrogen-driven genes expression induced by HAS2 overexpression in the MCF7 cell line through an ER-independent mechanism (Vanneste et al., 2017). To further analyze the characterization of the antagonism between estrogens signaling and HA turnover during breast cancer progression, we decided to study the impact of the increase in HA catabolism on estrogens signaling. Thus, ERα expression and estrogens responsiveness was studied in the ER + MCF7 breast cancer cell line cultured in the presence or absence of exogenous Hyal. Moreover, since HA catabolism is closely related to CD44 (Stern, 2004) and keeping in mind that CD44S plays a crucial role in EMT during breast cancer progression (Brown et al., 2011), a model of MCF7 cell line overexpressing CD44S was also developed and used to study the effect of HA catabolism on estrogen signaling.

2. Materials and methods

2.1. Antibodies and reagents

Mouse monoclonal anti-ERα (F-10), mouse monoclonal anti-CD44 (HCAM (DF-1485)) antibodies, RIPA lysis buffer system, goat anti-mouse IgG-HRP antibody, goat anti-mouse IgG-Texas Red and UltraCruz mounting medium were provided by Santa Cruz Biotechnology. Mouse monoclonal anti-β-actin antibody was provided by Millipore. The activation of MAPK and Akt signaling were respectively analyzed using PhosphoPlus® p44/42 MAPK (Thr202/Tyr204) and the PhosphoPlus® Akt (S473) Antibody kit respectively (Cell Signaling Technologies). Hyaluronidase from bovine testes, 4-methylumbelliferone, 17-β-estradiol, 4-hydroxytamoxifen, TriReagent, Sodium Dodecyl Sulfate (SDS), β-merceptoethanol, Tris-HCl, LY294002, BAY 11–7085, AG 1478, UO 126 and Bovine Serum Albumin (BSA) was obtained from Sigma Aldrich. Hyaluronan (Ultra low and high molecular weight) was provided by R&D systems. Blasticidin was provided by InVivogen. Plasmid vectors pWZL-CD44S and pWZL-GFP were provided by addgene. Trypsin/EDTA and Phosphate-buffered saline (PBS) was provided by PAN. Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT), deoxy Nucleotide Tri Phosphate (dNTP), RNasin, GoTaq qPCR master mix, Enhanced Chemiluminescence (ECL) and Random Primers were provided by Promega. Hybond ECL nitrocellulose membrane and hyperfilm ECL was provided by Amersham. Culture plates and flasks were obtained from Falcon.

2.2. Cell lines and cell culture

The human breast cancer cell lines MCF7 (ECACC 86012803) and MDA-MB-231 were maintained with Ham's F12/DMEM (Pan) (1:1) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Fisher Scientific) in a 5% CO_2 incubator at 37 °C. Cells were passaged when reaching confluence. To overcome any estrogenic activity, all experiments were carried out in phenol red-free DMEM (Pan) supplemented with 10% charcoal-stripped FBS.

2.3. Stable transfection

MCF7 cells were seeded in 6-well plates at 6×10^5 cells/well for 24 h to allow cells attachment. Cells were transfected with GenJet In Vitro DNA tranfection reagent (SignaGen Laboratories) according to the manufacturer's protocol. The ratio DNA:GenJet used was 1:3. MCF7 cells tranfected with pWZL-CD44S and pWZL-GFP were named MCF7 CD44S and MCF7 GFP, respectively. Forty eight hours after transfection, the cells were incubated in Ham's F12/DMEM – 10% FBS containing Blasticidin (10 μg/ml) to select for stable transfectants.

2.4. Crystal violet (CV) cell proliferation assay

Cells were cultured in 96-well plates at 4×10^3 cells/well for 24 h to allow cells attachment. To determine the number of cells after the treatment, a cell range (4×10^3; 8×10^3; 1.6×10^4; 3.2×10^4 cells/well) was performed in parallel for each experiment and was stained with CV at t = 0. MCF7 cells were incubated with or without hyaluronidase (100 UI/ml) for 24 h and then with or without estradiol (E2) (10^{-11} M), Hyal (100 UI/ml) and/or 4-OH tamoxifen (Tam) (10^{-6} M)