MOLECULAR ONCOLOGY 1 (2008) 413–424

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Androgens act synergistically to enhance estrogen-induced upregulation of human tissue kallikreins 10, 11, and 14 in breast cancer cells via a membrane bound androgen receptor

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ARTICLE INFO

Article history:
Received 30 October 2007
Received in revised form
28 December 2007
Accepted 1 January 2008
Available online 9 January 2008

Keywords:
Kallikreins
Breast cancer
Gene expression
Steroid hormones
Hormone-dependent gene expression
Hormone receptors
Cell signaling

ABSTRACT

The regulation of gene expression by steroid hormones plays an important role in the normal development and function of many organs, as well as in the pathogenesis of endocrine-related cancers, especially breast cancer. However, clinical data suggest that combined testosterone and estrogen treatments on post-menopausal women increase the risk of breast cancer. Experiments have shown that many, if not all kallikreins are under steroid hormone regulation in breast cancer cell lines. Their implication as prognostic and diagnostic markers has also been well-documented. Thus, we investigated the effect of combined hormone stimulation with androgens and 17β-estradiol on the ductal carcinoma cell line BT474. This cell line has been shown to be sensitive to both, androgens (secreting PSA) and estrogens (secreting a number of kallikreins including KLK10, 11, and KLK14). We found that PSA expression was downregulated upon combined hormone stimulation, confirming reports that estrogen can antagonize and block the activity of the androgen receptor. Upon analysis of estrogen-sensitive kallikreins 10, 11, and 14, all showed to be synergistically enhanced in their expression three- to fourfold, upon joint hormone treatment versus individual hormone stimulation. The enhancement is dependent upon the action of androgens as treatment with the androgen receptor antagonist cyproterone actetate normalized the expression of KLK10, 11, and KLK14 to estrogen-stimulation levels. The synergistic effects between estrogens and androgens on estrogen-sensitive genes may have implications on the role of the kallikreins in associated risk of breast cancer and progression.

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Non-standard abbreviations: KLK, kallikrein gene; KLK, kallikrein protein; PSA, prostate-specific antigen; DHT, dihydrotestosterone; AR, androgen receptor; ER, estrogen receptor; PGR, progesterone receptor; GR, glucocorticoid receptor; IGFBP4, insulin-like growth factor binding protein-4; ADAMTS-1, A Disintegrin And Metalloproteinase with ThromboSpondin repeats-1.

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doi:10.1016/j.molonc.2008.01.001

1. Introduction

Steroid hormones play a critical role in breast cancer development and have been associated with an increased epithelial cell proliferation and in turn facilitating malignant transformation. In particular, two sex hormones that have been very well characterized both *in vitro* and *in vivo* are estrogen and progesterone (Somboonporn and Davis, 2004a; Stein and McDonnell, 2006). The serum concentrations of these hormones together with their respective receptors are also used as epidemiological markers in assessing breast cancer risk. Studies have also shown that the direct action of these steroid hormones on different breast tissues is dependent upon their specific receptors. Another category of sex hormones that has been extensively studied in breast cancer in human and mice are androgens. Androgens have been shown to have both stimulatory and inhibitory actions on the growth of several breast cancer cell lines (Maggiolini et al., 1999; Hackenberg et al., 1988; Poulin et al., 1988; Zhou et al., 2000). However, their etiological role in breast cancer has been unclear. Unclear is whether the action of androgens is direct through their cognate receptor or via their metabolization into estrogen-like byproducts by aromatase activity. Also, recent studies suggest that subnormal levels of androgens may adversely affect a women's health, while on the other hand other studies indicate that supranormal levels may also have adverse effects on the female reproductive system including abnormal growth and tumorgenesis.

When women reach menopausal age, there is a decrease in endogenous levels of sex hormones, particularly testosterone and estrogen, and have been associated with menopausal symptoms. Clinical trials have demonstrated that the exogenous administration of these hormones can ameliorate these symptoms partially. However, there have been several studies that have associated endogenous elevated serum levels of estrogen and free testosterone hormone with breast cancer risk. This increased risk is of particular significance in postmenopausal women receiving HRT (Somboonporn and Davis, 2004a,b; Kaaks et al., 2005; Cummings et al., 2005; Cauley et al., 1999; Tworoger et al., 2005).

The molecular mechanism of the action of sex hormones is that they exert their effect by binding to their cognate hormone receptor. Upon binding to the receptor, the hormone–receptor complex translocates into the nucleus, binds to DNA *cis*-elements known as hormone response elements (HREs) in the upstream proximal promoter, and interacting with several other coactivating proteins and the general transcriptional machinery to modulate transcriptional activation. The consensus HRE sequence consists of a palindromic sequence separated by a unique nucleotide sequence. Hormone receptors, in particular the glucocorticoid, androgen and progesterone receptors (GR, AR and PGR, respectively) recognize very similar DNA *cis*-elements, however, the estrogen receptor (ER) binds to a quite unique sequence (Klinge, 2001; Aranda and Pascual, 2001; Claessens et al., 2001). Therefore, the sensitivity/expression of a particular hormone-dependent regulated gene in a cell line to any given steroid hormone is dependent upon both the presence of the hormone receptor and consensus HRE binding sites. By far, the gene whose regulation by steroid hormones has been most thoroughly studied is the human tissue kallikrein gene, *prostate-specific antigen* (*PSA*). The*PSA* gene possesses three androgen response elements (ARE-I, ARE-II, and ARE-III). ARE-I and ARE-II were identified in the upstream promoter region (−170 bp and −400 bp), functionally tested and found to be active in LNCaP, a prostate cancer cell line (Cleutjens et al., 1996; Cinar et al., 2004). ARE-III was found at −4316 bp, which induced a dramatic increase in *PSA* transcription, in comparison to ARE-I and ARE-II (Cleutjens et al., 1997). AREs have been found in other genes, including other members of the kallikrein gene family. We are currently in the process of elucidating hormone responsive elements for other kallikreins. More recently, literature is accumulating for non-genotropic actions of steroid hormones via another category of hormone receptors, which are associated with the plasma membrane. Instead the actions of these steroid hormone receptors are characterized by activation of a variety of signal transduction pathways including, MEK/ERK, PI3K/AKT, and JNK pathways (Zivadinovic and Watson, 2005; Peterziel et al., 1999; Kang et al., 2004; Papakonstanti et al., 2003; Stoica et al., 2003a).

All 15 kallikrein genes show differential expression patterns in many cancers at the mRNA and protein levels and many kallikreins have been examined as prognostic indicators in breast cancer including, *PSA*, *KLK5*, *6*, *10*, and *KLK14* (Yousef et al., 2002a,b; Sidiropoulos et al., 2005; Pampalakis and Sotiropoulou, 2006; Borgono et al., 2003; Yu et al., 1996; Obiezu and Diamandis, 2005; Paliouras et al., 2007). Previous studies have found that there is a close association between steroid hormone stimulation of breast cancer cell lines and coordinated kallikrein gene expression (Borgono et al., 2003; Luo et al., 2000; Paliouras and Diamandis, 2006a; Magklara et al., 2000). However, it has never been examined if the expression profiles would change upon multiple hormone stimulations. Therefore, would significant changes in kallikrein gene expression be of clinical importance within the context that HRT with estrogen and testosterone and increases in breast cancer risk? Thus, in this paper we examined a number of androgen and estrogen hormone-regulated kallikrein genes in the breast cancer cell line BT474, to determine if these two steroid hormones can act synergistically to enhance kallikrein gene expression.

2. Materials and methods

2.1. Cell lines

The breast cancer cell line BT474, used in the following experiments was obtained from the American Type Culture Collection (ATCC), Rockville MD, and was selected as to their well-defined kallikrein expression.

2.2. Steroids and inhibitor compounds

All steroid hormones, the steroid antagonist cyproterone acetate, and BSA-conjugated testosterone (testosterone 3-(*O*-carboxymethyl)oxime:BSA) were obtained from Sigma Chemical Co., St. Louis, MO. The aromatase inhibitor xanthenone (4-(imidazolylmethyl)-1-nitro-9*H*-9-xanthenone) was