Coordinated Hormone Regulation of Multiple Kallikreins in Breast Cancer Cell Lines

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ABSTRACT

The regulation of gene expression by steroid hormones plays an important role in the normal development and function of many organs as well in the pathogenesis of endocrine-related cancers. Experiments have shown that many, if not all kallikreins are under steroid hormone regulation, particularly in breast cancer cell lines. Several breast cancer cell lines, representing both benign and metastatic origin were selected for hormonal studies, including: BT-20, BT-474, T-47D, MCF-7, MDA-MB-468, and MDA-MB-231. Following hormone stimulation, kallikrein (hK) expression for PSA (hK3), hK5, hK6, hK8, hK10, hK11, hK13, and hK14 were analyzed at the RNA level via semi-quantitative RT-PCR and by ELISA to measure secreted protein production. BT-20 and MDA-MB-231 failed to show any expression of any of the selected hKs, whereas MDA-MB-468 showed high hK expression regardless of hormone treatments. Hormone-specific modulation of expression was seen for several kallikreins in BT-474, MCF-7, and T-47D. hK6 was specifically upregulated upon estradiol treatment in all three cell lines. PSA expression was sensitive to dihydrotestosterone (DHT) and Norgestrel stimulation in BT-474 and T-47D, supporting the requirement of specific androgen response elements (AREs) for its activation. We found that hK10, 11, 13 and 14 were specifically upregulated by DHT in T-47D and by estradiol in BT-474 cell lines. Bioinformatic analysis of upstream proximal promoter sequences for these hKs did not identify any recognizable HREs, suggesting that the coordinated activation of these four hKs represents a unique expression "cassette," utilizing a common hormone-dependent mechanism, likely different from classical HREs. Using reporter gene-promoter deletion constructs, the identification of cis-elements regulating the expression of hK5, 6, 10 and hK11 is currently being investigated in both hormone-sensitive and non-sensitive cell lines. Our conclusion that groups of human hKs are coordinately expressed, by a hormone-dependent process, provides further support to the notion that hKs participate in cascade enzymatic pathways in hormone-dependent tissues.

INTRODUCTION

Steroid hormones, in particular estrogens, play an essential role in breast cancer development and their involvement in breast cancer tumorgenesis is associated with an increase in breast epithelial cell proliferation, thus facilitating malignant transformation . All 15 kallikrein genes show differential expression patterns in many cancers at the mRNA and protein levels and many kallikreins being assessed as prognostic indicators in breast cancer. Previous studies have found that there is a close association between steroid hormone stimulation in cancer cell lines and kallikrein gene expression.

Steroid hormones exert their effect by binding to their cognate hormone receptor. Upon binding to the receptor, the hormone-receptor complex translocates into the nucleus and activates gene transcription via binding to specific DNA sequences known as hormone response elements (HREs). HREs are usually found in upstream promoter regions and recruit coactivators/corepressors to the general transcriptional machinery to modulate transcriptional activation. Hormone receptors, in particular the androgen and progesterone receptors (AR and PR, respectively) recognize very similar DNA *cis*-elements, however, the estrogen receptor (ER) binds to a quite unique sequence. Therefore, the sensitivity/expression of a particular kallikrein 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 kallikrein whose regulation by steroid hormones has been most thoroughly studied is *KLK3 (PSA)*. Initially, two androgen response elements (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. An additional ARE was found at -4,316 bp, which induced a dramatic increase in *KLK3* transcription, in comparison to ARE-I and ARE-II. The androgen-dependent expression of PSA and hK2 represents the "classical" known regulatory mechanism of members of the kallikrein gene family. Along with androgen sensitivity in prostate cancer cell lines, *KLK2* and *KLK3* expression is also up-regulated by androgens and progestins in the breast cancer cell lines BT-474, T-47D and MFM 223. *KLK4* was also found to be up-regulated by androgens in the prostate cancer cell line LNCaP. Putative AREs have been identified in the immediate upstream promoter region of *KLK4*, however, they have not been functionally tested. Such similarities could account for the shared expression patterns seen between these three genes, especially in the androgen sensitive organ, the prostate

OBJECTIVES

Until now hormone-dependent kallikrein gene expression studies have either been limited to individual kallikrein genes or to specific cancer cell lines. There is now evidence indicating presence of multiple kallikreins in breast tumor-associated biological fluids and expression levels that correlate with steroid hormone receptors. Therefore, we selected several breast cancer cell lines representing benign (BT-20), solid tumors (BT-474) and metastatic variants (T-47D, MCF-7, MDA-MB-468, and MDA-MB-231) to investigate the hormone-dependent regulation of multiple kallikrein family members.

RESULTS

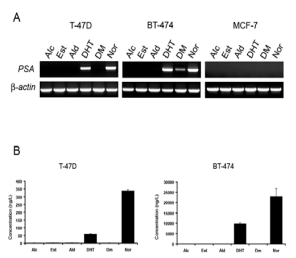


Figure 1. PSA expression profile in breast cancer cell lines. A. RT-PCR analysis of *PSA* expression in hormone responsive cell lines T-47D, BT-474, and MCF-7. *PSA* shows specific DHT and norgestrel sensitive upregulation in T-47D and BT-474, but not in MCF-7. Actin expression was used as a control of RT-PCR analysis, and for subsequent other RT-PCRs shown in this article. B. PSA protein production from T-47D and BT-474 was quantified by ELISA in T-47D and BT-474 and found to be similar to the RNA expression profile produced upon hormone treatments. Alc, ethanol; Est, 17b-estradiol; Ald, aldosterone; DHT, 5a-dihydrotestosterone; Dm, Dexamethazone; Nor, Norgestrel. Hormones were used at a final concentration of 10nM.

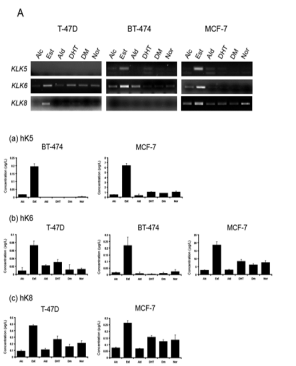


Figure 2. Estradiol hormone specific kallikrein expression. A. RT-PCR analysis of *KLK5*, *KLK6*, and *KLK8* show specific and selective upregulation by estradiol in all three hormone sensitive breast cancer cell lines used in this study. *KLK6* is upregulated in all three lines, but *KLK5* and *KLK8* expression is limited to two lines and shown. B. Protein production of hK5, hK6, and hK8 as measured by ELISA assays, (a) hK5, (b) hK6, and (c) hK8.

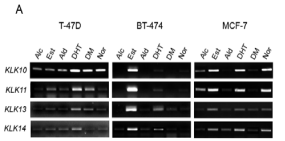
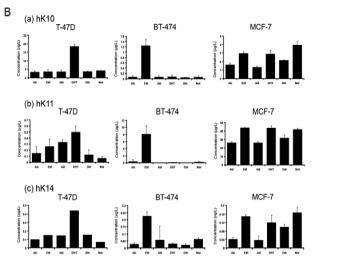


Figure 3. "Cassette" kallikrein expression. A. *KLK10, KLK1, KLK13,* and *KLK14* mRNAs are upregulated by different hormones in T-47D, BT-474, and MCF-7. These kallikreins are upregulated by androgen in T-47D, estradiol in BT-474 and sensitive to all three sex hormones (Est, DHT, Nor) in MCF-7. B. Protein expression profiles are parallel to the observed RNA expression patterns, (a) hK10, (b) hK11, and (c) hK14.



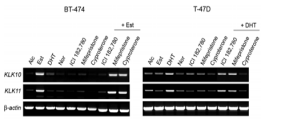
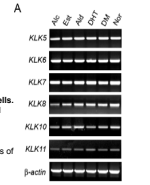


Figure 4. *KLK10* and *KLK11* are repressed by respective hormone inhibitors in T-47D and BT-474 upon hormone stimulation. RT-PCR performed on RNA extracted from hormone plus antagonist treated hormone stimulated cells, reveal that the hormone specific upregulation of *KLK10* and *KLK11*, although stimulated by different hormones in T-47D (DHT) and BT-474 (17b-estardiol), expression is still dependent upon their respective hormone receptors. Hormone receptor antagonists were used at a final concentration of 100 nM.



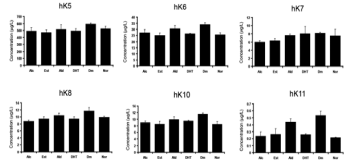


Figure 5. Dysregulated hormone-independent kallikrein expression in MDA-MB-468 cancer cells. A. RT-PCR gels of kallikreins 5, 6, 7, 8, 10, and 11 indicare that kallikreins are no longer under the regulation of steroid hormones. b-actin RT-PCR is used as a control for semi-quantitative RT-PCRs performed in these experiments. B. ELISA assays of these kallikreins correlate the with RT-PCR data.

CONCLUSION

Table 1. Summary of Human Tissue Kallikrein Hormone Regulation

KLK	Cell Line	Quantitation Method	Hormone Sensitivity
PSA	BT-474	RT-PCR, ELISA	Norgestrel>DHT
	T-47D	RT-PCR, ELISA	Norgestrel>DHT
KLK5	BT-474	RT-PCR, ELISA	Estradiol
	MCF-7	RT-PCR, ELISA	Estradiol
KLK6	BT-474	RT-PCR, ELISA	Estradiol
	T-47D	RT-PCR, ELISA	Estradiol
	MCF-7	RT-PCR, ELISA	Estradiol
KLK8	T-47D	RT-PCR, ELISA	Estradiol
	MCF-7	RT-PCR, ELISA	Estradiol
KLK10	BT-474	RT-PCR, ELISA	Estradiol
	T-47D	RT-PCR, ELISA	DHT
	MCF-7	RT-PCR, ELISA	Estradiol, DHT, Norgestrel
KLK11	BT-474	RT-PCR, ELISA	Estradiol
	T-47D	RT-PCR, ELISA	DHT
	MCF-7	RT-PCR, ELISA	Estradiol, DHT, Norgestrel
KLK13	BT-474	RT-PCR	Estradiol
	T-47D	RT-PCR	DHT
	MCF-7	RT-PCR	Estradiol, DHT, Norgestrel
KLK14	BT-474	RT-PCR, ELISA	Estradiol
	T-47D	RT-PCR, ELISA	DHT
	MCF-7	RT-PCR, ELISA	Estradiol, DHT, Norgestrel

DISCUSSION:

This study, on the hormonal regulation of kallikrein gene and protein expression in several breast cancer cell lines, has allowed for the expansion to three kallikrein research fields. First, understanding the underlying mechanism by which kallikreins are regulated. Traditional models of the hormonal regulation of these genes need to be modified since we observed differential hormone sensitivity (androgens vs. estrogens) of the same kallikreins in different cell lines. Second, the link of kallikrein expression profiles as observed between the relatively benign BT-20 cells, versus hormone-dependent cell lines, and the metastatic MDA-MB-468 cell line, to clinical multi-parametric kallikrein analysis of different tumor stages. Non-kallikrein related studies of estradiol stimulated MCF-7 cells show that there is an increase in cell proliferation and growth, associated with specific changes in gene expression such as increases in transcriptional expression of proto-oncogenes. Finally, what role do the kallikreins play in tumor progression? Multiple kallikreins, such as *KLK5, KLK6,* and *KLK14* have already been implicated in breast cancer progression as the idea that kallikrein enzymes participate in proteolytic cascade pathways originated from the discovery of putative substrates combined with their expression patterns in different tissues.