Intracellular signaling pathways regulate expression of Prostate Specific Antigen and other kallikreins in human breast cancer cells

Miltiadis Paliouras and Eleftherios P. Diamandis

Samuel Lunenfeld Research Institute, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada

MOUNT SINAI HOSPITAL

The Samuel Lunenfeld Research Institute

Advanced Centre for Detection of Cancer

www.acdclab.org

ABSTRACT

Human tissue kallikreins (KLKs) in breast cancer cell lines show both hormone-dependent and –independent expression, however the regulatory pathways which ultimately lead to gene activation have not been dissected. This study suggests that certain signal transduction pathways may influence regulation of both hormone-dependent and -independent KLK gene expression. The hormone-sensitive breast cancer cell line, T47D, and one hormone – independent cell line, MDA-MB-468, were chosen to study the involvement of the intracellular signaling pathways in regulating kallikrein gene expression. PSA, KLK10 and KLK11 are upregulated upon androgen (dihydrotestosterone-DHT) stimulation in T47D cell line. The expression of these KLKs was repressed by the MEK1/2 inhibitor U0126 in the presence of the hormone, thus implicating the RAS/ERK signaling pathway in regulating hormone-dependent KLK gene activation. Furthermore, treatment with the PI3K inhibitor Wortmannin also resulted in decreased expression of these kallikreins in the presence of DHT. However, selective chemical inhibitors against JNK, p38 MAPK, JAK, NFkB, and GSK3b pathways showed differential regulation of the above KLKs, with PSA and KLK11 showing parallel regulation patterns versus KLK10. KLK5 and KLK6 whose expression is independent of hormone activity in MDA-MB-468 cells also showed differential selectivity in their regulation of these upon inhibitor treatments. Furthermore, analysis of inhibitor treated cells for changes in transcription factor expression showed that specific factors were altered in a pattern parallel to kallikrein expression. We conclude that the hormone-specific upregulation of PSA, KLK10 and KLK11 and the dysregulated expression of KLK5 and KLK6 in breast cancer cell lines are dependent on major intracellular signaling pathways, as well as specific transcription factors, providing a new dimension to the regulation of these cancer-related genes.

INTRODUCTION

Our project is to elucidate the intracellular pathways involved in both hormone-dependent and hormone-independent genetic regulation of kallikreins in prostate and breast cancer. Unlike, in other endocrine related cancers such as ovarian cancer, kallikrein levels from biological fluids and tissue biopsies of these two cancers, show similar expression profiles and related to stage and type. Therefore, a similar mechanism may be involved that would regulate kallikreins gene expression in both prostate and breast cancer. Genes can be regulated by a variety of mechanism; transcriptional, RNA processing, and translation. Each mechanism is unique, but none of these steps of gene regulation is the sole rate-limiting step in gene expression. The processes regulating gene expression, are interdependent upon various cellular processes especially, signal transduction pathways. Cell signaling pathways are utilized by all steps of gene expression, and control the activities of various receptors, kinases, phosphatases, and co-factors/co-activators/co-repressors for individual and common genes. In turn, these pathways and the resulting gene expression contribute to cellular differentiation, development, and disease progression. In order to perform this project, we have utilized selective inhibitor treatment approach to specific cell signaling pathways that have been implicated in Prostate Specific Antigen (PSA) regulation in prostate cancer cell lines, to analyze the expression profiles of other coordinately expressed kallikreins in both prostate and breast cancer cell lines.

Overall, carcinogenesis is a complex process that is a result in alterations in gene expression. One of the goals of cancer research is to identify these alterations and to determine its effects on tumor phenotype. All 15 kallikrein genes show differential expression patterns in many cancers (primarily endocrine or hormone-related cancers) at the mRNA and protein levels. Identifying gross genetic aberrations within the kallikrein locus of diseased tissues that show kallikrein dysregulation is also currently being examined. Palioura*s et al.* 2007 highlights the kallikreins that are associated with breast and prostate cancers. 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. A number of experiments in endocrine-related tissues, in both cell culture and in vivo, have shown that most, if not all KLKs are under steroid hormone regulation.

More recently, several studies suggest the possibility that signal transduction pathways may influence the hormonal regulation of kallikrein gene expression. The traditional understanding of androgen receptor (AR) induced gene expression simply relied on the binding of the hormone to the receptor and binding of the complex to the ARE upstream of the gene. The AR has been shown to be activated by several pathways including MEK through RAS pathway, AKT kinases and PKC which sensitizes the receptor to low circulating levels of androgen. Using RAS effector-loop gain-of-function RAS mutant stable cell lines, it has been shown that constitutive MEK activation can hyper-induce PSA protein expression in LNCaP cells under normal levels of androgen.

We are now realizing that many signal transduction pathways are playing a role in regulating kallikrein gene expression. It has been found that approximately 30% of all breast cancers either have a deletion or mutation in the gene encoding the tumor suppressor protein *phosphatase and tensin homologue deleted from chromosome 10 (PTEN).* PTEN is a negative regulator of AKT function, resulting in increases in cell growth and proliferation. Therefore, it is also currently being investigated whether kallikrein gene expression can be regulated through AKT function. It is worth noting that *PTEN* deficient cells are no longer sensitive to current therapeutics agents such as CCI-779 and Tamoxifen.

METHODS

Chemical Inhibitors to cell signaling pathways

Inhibitor	Signaling Pathway	Concentration*
U0126	MEK1/2	5 μM
Wortmannin	PI3K	200 nM
Parthenolide	NFκB	10 μM
SP600125	JNK	30 μM
SB203580	p38 MAPK	10 μM
AG490	JAK/STAT	20 μM
α-4-Dibromoacetophenone	GSK3β	10 μM
(Z,E)-5-(4-Ethylbenzylidine)-2-thioxothiazolidin-4-one	c-MYC/MAX	15 μM

*Concentrations used for our inhibitor treatments in all cell lines.

RESULTS

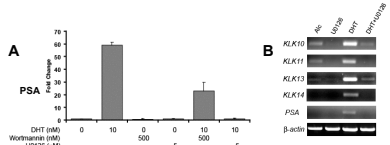

Figure 1. Changes in KLK expression upon MEK1/2 and PI3K inhibition of the hormone sensitive cell line T47D. A. T47D cells were stimulated with DHT in the presence of either the MEK1/2 inhibitor U0126 or the PI3K inhibitor Wortmannin and changes in PSA and KLK10 protein levels were analyzed by ELISA. **B.** RT-PCR analysis of *PSA, KLK10, 11, 13*, and *KLK14*.

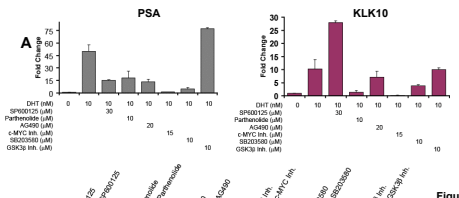

Figure 2. Changes in KLK expression profiles upon inhibition of alternative intracellular signaling pathways of T47D. A. Changes in PSA and KLK10 protein levels. **B.** RT-PCR analysis of *PSA, KLK10* and *KLK11*.

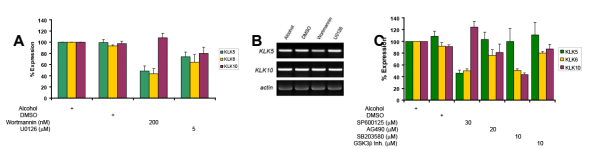

Figure 3. Analysis of KLK5, 6, and KLK10 in the hormone-independent breast cancer cell line MDA-MB-468. A. Changes in protein expression profiles upon inhibitor treatments. **B.** RT-PCR analysis of *KLK5* and *KLK10* mRNA. **C.** Analysis of KLK5, 6, and KLK10 I MDA-MB-468 upon inhibition of alternative intracellular signaling pathway.

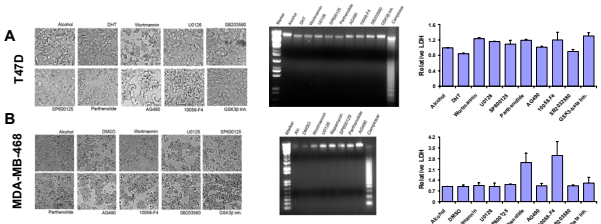

Figure 4. Analysis of changes in cell viability upon selective inhibitor treatments. Analyzed changes in cell morphology, DNA apoptosis and LDH. **A.** T47D. **B.** MDA-MB-468.

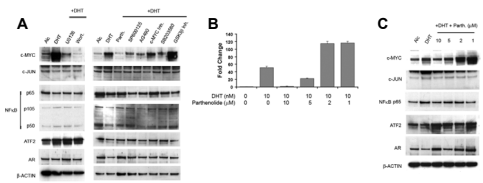

Figure 5. Analysis of transcription factor expression upon selective inhibitor treatments of androgen stimulated T47D cells. A. Western blot analysis of a select number of transcription factors whose activity is affected by the chemical inhibitor used in the study (not shown is c-FOS, however there are no changes in the expression levels upon inhibitor treatmenst. **B.** PSA is upregaulted by low dose levels of the NFκB inhibitor Parthenolide in T47D cells. A similar expression profile is seen for KLK11 at low concentrations of Parthenolide (data not shown) **C.** Western blot analysis of various transcription factors upon Parthenolide treatments. c-MYC shows a parallel expression profile to PSA at low levels of Parthenolide.

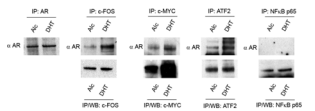

Figure 6. The Androgen receptor co-immunoprecipitates with a number of transcription factors. We analyzed whether the androgen receptor interacts with various transcription factors that were targeted in our studies, by co-immunoprecipitations. The androgen receptor co-IP'd with c-MYC, c-FOS, and ATF2 and c-JUN (data not shown). Although, it appears that NFκB is required for *PSA, KLK10* and *KLK11* gene expression it did not co-IP the androgen receptor.

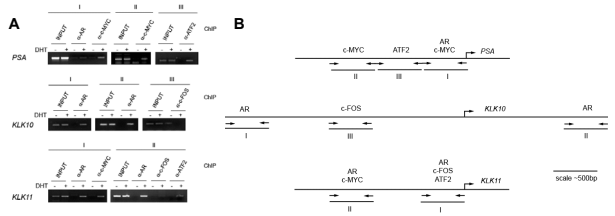

Figure 7. Chromatin Immunoprecipations of PSA, KLK10, and KLK11. A. Several transcription factors are bound to promoter regions of PSA, KLK10 and KLK11. Specifically, we have identified two c-MYC binding sites in PSA proximal promoter as well as a c-MYC binding site in KLK11, but not KLK10. We have also identified androgen response elements for both KLK10 and KLK11. **B.** Schematic diagram of the location of the ChIP primers for the three genes analyzed. ChIP PCR primers were designed to amplify approximately 500bp fragment.

DISCUSSION and CONCLUSIONS

Summary of signaling pathways and kallikrein gene expression

KLK (cell line)	Signaling Pathway: MEK1/2	PI3K	NFκB	JNK	JAK/STAT	p38MAPK	GSK3β
PSA (T47D)	positive	positive	positive	positive	positive	positive	positive
KLK5 (MDA-MB-468)	NR	positive	ND	positive	NR	NR	NR
KLK6 (MDA-MB-468)	NR	positive	ND	positive	NR	positive	NR
KLK10 (T47D)	positive	positive	positive	negative	NR	positive	NR
KLK11 (T47D)	positive	positive	positive	positive	positive	positive	positive

NR- not required
ND- not determined (concentrations of inhibitors caused cell death)