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Short Communication

Glucocorticoid receptor-mediated expression of kallikrein 10 in human breast cancer cell lines

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Abstract

Using the breast cell lines MCF-10A, MDA-MB-468 and T-47D, we investigated the role of various glucocorticoids in regulating human kallikrein 10 expression. We found that increased concentrations of glucocorticoids decreased KLK10 expression in MCF-10A and increased KLK10 expression in MDA-MB-468 and T-47D cells. Stimulation of the cell lines using other steroid hormones did not yield any difference in KLK10 expression in MCF-10A and MDA-MB-468 cells, suggesting that regulation of KLK10 occurs primarily through glucocorticoids. However, T-47D cells expressed higher levels of KLK10 upon dihydrotestosterone stimulation. Blocking the glucocorticoid receptor (GR) demonstrated that the mechanisms of induction and repression are different in the three cell lines studied. Taken together, our results suggest an alternative mode of KLK10 regulation – by glucocorticoids via GR-dependent mechanisms.

Keywords: betamethasone; cell culture; dexamethasone; differential regulation; mifepristone; steroid hormones.

Despite tremendous progress in our understanding of the molecular basis of many diseases, cancer continues to be a major cause of morbidity and mortality among men and women. Breast cancer is a major cause of death among women with solid tumors in North America (van Diest et al., 2004). It is a disease of the middle and late ages of life, as 75% of breast cancer is diagnosed in women over the age of 50. While breast cancer is less common at a young age, younger women tend to have a more aggressive form of the disease than older women. The mortality rate has declined in recent years, in part due to both improved treatments and early detection; however, the incidence has steadily increased over the past three decades. Metastasis is the main cause of treatment failure and death in patients with breast cancer.

Members of the human kallikrein family (*KLK* gene, KLK protein) have been implicated in the process of carcinogenesis and the application of kallikreins as biomarkers for diagnosis and prognosis is currently being investigated. Kallikreins are secreted enzymes that encode for trypsin- or chymotrypsin-like serine proteases. They consist of a family of 15 genes clustered in tandem on chromosome 19q13.3–4 (Borgono and Diamandis, 2004). Prostate-specific antigen (PSA; KLK3), belonging to the family of human tissue kallikreins, and human kallikrein 2 (KLK2) currently have important clinical applications as prostate cancer biomarkers (Rittenhouse et al., 1998).

Human kallikrein 10 (KLK10), also known as normal epithelial cell-specific-1 (NES1), was identified by Liu et al. (1996) by subtractive hybridization between normal and immortalized breast epithelial cell lines. KLK10 is abundantly expressed in the sex organs, including breast, ovary, prostate and testis (Yousef and Diamandis, 2001). The physiological functions and substrates of KLK10 are still unclear, although *KLK10* has been postulated to be a tumor suppressor gene (Goyal et al., 1998). Previous studies, including *in silico* analyses, have shown that KLK10 is down-regulated in breast cancer cell lines, thus implicating this gene in breast cancer progression and pathogenesis (Dhar et al., 2001; Yousef et al., 2004). However, another study found that there was no correlation between KLK10 levels and tumor size, grade and nodal status, and that high KLK10 levels in breast tumors were associated with tamoxifen resistance (Luo et al., 2002). Given that KLK10 is expressed predominantly in sex organs, it is reasonable to speculate that the expression of KLK10 in these organs is modulated by sex hormones. In fact, the majority of the kallikrein family members are modulated by multiple steroid hormones (Yousef and Diamandis, 2001).

Steroid hormone receptors are intracellular receptors mediating signal transduction and transcriptional activity by steroid hormones. They are part of the nuclear receptor family, which can be broadly grouped according to mechanism into type I and type II receptors (Novac and Heinzel, 2004). Type I receptors include the sex hormone receptors (androgen, estrogen and progesterone), glucocorticoid receptor (GR) and the mineralocorticoid receptor. A biological response is influenced by the amount of hormone available, the available receptor population, the dissociation rate of the hormone-receptor complex from the specific DNA site, and the replenishment of the receptor population (Aranda and Pascual, 2001).

The GR is a ligand-activated intracytoplasmic transcription factor that interacts with high affinity with cor-

tisol and other glucocorticoids (GCs) (Fruchter et al., 2005). The number of genes per cell directly regulated by GCs is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors and coactivators (Adcock et al., 2004). GRs are ubiquitous in cancer cells and have been linked to signal transduction pathways pertinent to their growth, defense and apoptosis (Lu et al., 2005). Some of the established actions of GCs include regulation of metabolism, inhibition of inflammation and the immune system, and suppression of bone formation (Fruchter et al., 2005). GCs play a major role as anti-inflammatory agents through repression of inflammatory and immune genes by interacting with NF-κB and AP-1 (Adcock et al., 2004). In the treatment of patients with solid tumors, co-administration of GC with other anti-cancer drugs is a common clinical practice to prevent drug-induced allergic reaction or nausea (Lu et al., 2005). Some studies have shown that GCs are growth-suppressive in mammary cancer cells (Wan and Nordeen, 2003). Other studies have documented GC-like effects of progesterone in various tissues (Leo et al., 2004). Thus, without specific synthetic steroids that selectively activate one receptor and not the other, it is not easy to differentiate between GR and progesterone receptor (PR)-mediated effects when the two receptors are co-expressed in the same tissue, since GR and PR share a substantial number of common target genes (Cleutjens et al., 1997).

Our laboratory has previously published ELISA data on kallikrein expression in various breast cancer cell lines (Luo et al., 2003; Paliouras and Diamandis, 2007). Based on the results from this study and previous unpublished data, it was known that MCF-10A, T-47D and MDA-MB-468 cells express KLK10 without the need for hormonal stimulation. Other breast cancer cell lines express KLK10, but only on stimulation with other steroid hormones. For example, BT474 and MCF7 cells, two well-studied breast cancer cell lines, express KLK10 on estradiol stimulation for the former and with estradiol, dihydrotestosterone (DHT), and norgestrel for the latter. Thus, we decided to focus on only those cell lines that express KLK10 without the need for hormonal stimulation (MCF-10A, T47-D and MDA-MB-468) for further investigation regarding GC regulation.

Previous studies in our laboratory have shown that KLK10 expression is up-regulated by estradiol in certain breast cancer cell lines such as BT474 (Luo et al., 2000). At the mRNA level, using RT-PCR we investigated the regulation of *KLK10* in three breast cell lines by the GC dexamethasone. Since alcohol or DMSO was used to dissolve the hormones, alcohol or DMSO-treated cells were used as controls. On GC stimulation, differential expression of *KLK10* was observed in specific cell lines (Figure 1). In MCF-10A cells, an immortalized but non-tumorigenic cell line, 10^{-6} M dexamethasone drastically reduced *KLK10* expression compared to the control-treated cells. In MDA-MB-468 cells, a metastatic breast cancer cell line, the same concentration of dexamethasone increased the expression of *KLK10* compared to control-treated cells. Given the short incubation period (48 h), it was known that T-47D cells must be treated with 10^{-8} M DHT to stimulate *KLK10* expression. Therefore, for T-47D cells, we stimulated all conditions with DHT and

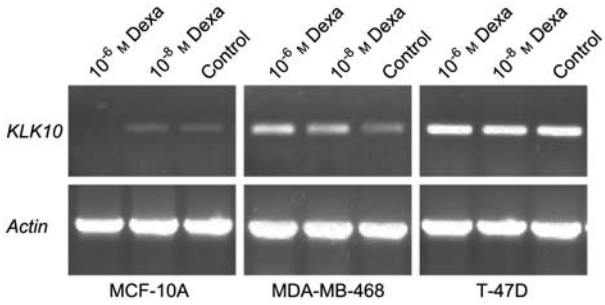

Figure 1 *KLK10* mRNA levels in breast cancer cell lines upon glucocorticoid stimulation.
RT-PCR analysis of *KLK10* expression in MCF-10A, MDA-MB-468 and T-47D cell lines when stimulated with control (DMEM or RPMI 1640 media alone), or 10^{-6} or 10^{-8} M dexamethasone (dexa). All steroid hormones were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Total RNA was extracted from lysates of dexamethasone-treated cells (48-h stimulation in 6-well plates) using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Samples of 5 μg of total RNA were reverse-transcribed into cDNA with an oligo-dT primer using Superscript II reverse transcriptase (Invitrogen). The final volume was 20 μl. PCR was performed for *KLK10* and *actin* in 50 μl of reaction mixture. The same PCR conditions were utilized for both *actin* and *KLK10* gene amplification. The primers for amplifying *KLK10* were 5′-GGA AAC AAG CCA CTG TGG GC-3′ (forward) and 5′-GAG GAT GCC TTG GAG GGT CTC-3′ (reverse). cDNAs were amplified using Hotstar *Taq* DNA polymerase (Qiagen, Valencia, CA, USA) with 25 cycles each. *Actin* expression was used as a control.

examined the effect of dexamethasone treatment. In these cells, no significant differences in expression levels were observed between the control and treatment groups for *KLK10*. It is possible that DHT stimulation of this cell line induced expression of *KLK10* and therefore the effect of GCs was not observed.

We then examined the effect of GCs on KLK10 expression at the protein level using an ELISA specific for KLK10. To maintain consistency among the three different cell lines used, all cells were stimulated in RPMI 1640 media containing charcoal/dextran-treated fetal bovine serum (FBS) to remove endogenous steroid hormones. Concentrations of 10^{-8} and 10^{-6} M were used for this study. As observed at the mRNA level, in MCF-10A cells the higher concentration of various GCs (dexamethasone, betamethasone, cortisol, prednisone and corticosterone) decreased the levels of KLK10 to almost zero (Figure 2A). Cortisol is the standard of comparison for GC potency. In general, the potency of GCs is: dexamethasone>betamethasone>prednisone>corticosterone>cortisol (Zoorob and Cender, 1998). Dexamethasone followed by betamethasone treatment of MCF-10A cells resulted in a more potent decrease in KLK10 compared to the other GCs tested, thus correlating with its potency. In MDA-MB-468 cells, dexamethasone treatment was more potent in stimulating KLK10 expression (Figure 2B). To maintain the consistency of the experimental conditions for all three cell lines, T-47D cells were not stimulated with DHT to increase basal levels of KLK10. Since the cells were incubated for 5 days, quantifiable levels of KLK10 were obtained from T-47D. Similar to MDA-MB-468, increased concentrations of GCs appeared to increase the protein expression of KLK10 (Figure 2C).

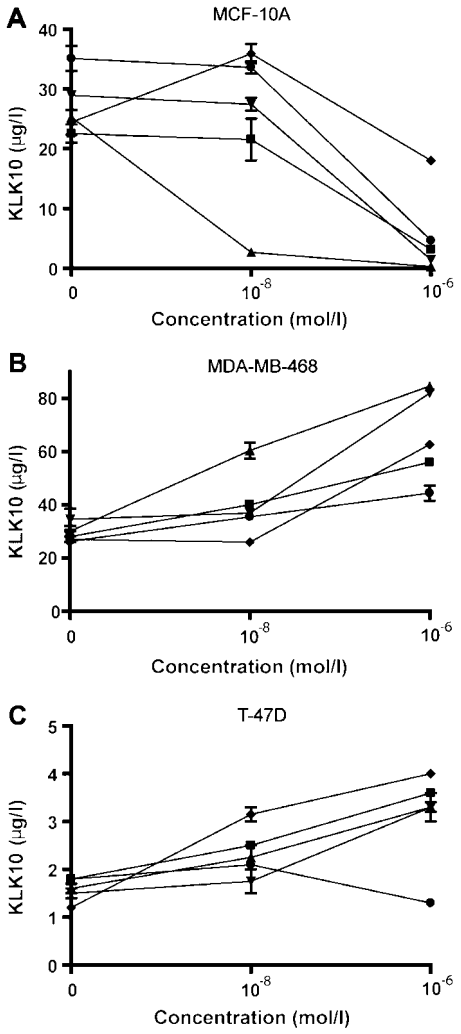

Figure 2 KLK10 protein production in breast cancer cell lines upon glucocorticoid stimulation.
The concentration of KLK10 was quantified with a KLK10-specific non-competitive immunoassay developed in our laboratory (Luo et al., 2001) in (A) MCF-10A, (B) MDA-MB-468 and (C) T-47D cells after stimulation with 10^{-8} and 10^{-6} M concentrations of betamethasone (BM; ■), dexamethasone (DM; ▲), cortisol (C; ▼), prednisone (PD; ♦) and corticosterone (CS; ●). Zero on the x-axis represents cells treated with DMSO alone.
The breast cell lines MCF-10A, MDA-MB-468 and T-47D were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). All tissue culture reagents were obtained from Gibco (Gaithersburg, MD, USA). Cells were seeded at a density of 250 000 in their respective culture media (according to ATCC) into 6-well plates (2 ml). Two days later, the medium was discarded and the cells were rinsed twice with 1× phosphate-buffered saline (PBS). Then 2 ml of phenol-red-free RPMI containing charcoal/dextran-treated FBS was added (devoid of endogenous steroid hormones). At this time, cells were treated with control (DMSO or alcohol), or 10^{-8} or 10^{-6} M of the various GCs. The cells were incubated for an additional 5 days, after which the conditioned media were collected for KLK10 measurements. All stimulations were performed in duplicate and each experiment was repeated multiple times to ensure reproducibility.

However, stimulation of T-47D cells with corticosterone showed a decrease rather than an increase in KLK10 expression. Given that the potency of corticosterone is relatively low compared to dexamethasone and betamethasone, this observation is not surprising. Thus, these results were consistent with the RT-PCR data presented above for MCF-10A and MDA-MB-468, suggesting differential regulation of KLK10 expression by various GCs at both the mRNA and protein levels. For T-47D cells, there was no change in expression of *KLK10* at the mRNA level (which could be attributed to DHT stimulation), but a change at the protein level was observed. Comparison between the mRNA and protein data for this cell line cannot be made because of the different experimental conditions used.

Next, we investigated whether other steroid hormones, in addition to GCs, had a similar effect in differentially regulating KLK10 expression. In MCF-10A cells, stimulation with 10^{-8} M alcohol (control), aldosterone, norgestrel, DHT, and estradiol did not yield a significant difference in the expression of KLK10 (Figure 3A). Consistent with our previous findings, only dexamethasone treatment resulted in a dramatic decrease in KLK10 in this cell line. Hormonal stimulation of MDA-MB-468 and T-47D cells with these steroid hormones has previously been reported by our laboratory; Paliouras and Diamandis (2007) found that in MDA-MB-468 cells, KLK10 expression did not differ among the hormones (10^{-8} M) tested, while only DHT treatment increased KLK10 expression in T-47D cells. In addition, we investigated whether increasing the concentration of the other steroid hormones would elicit differential expression. The three cell lines were stimulated with 10^{-8} and 10^{-6} M DHT and estradiol. Consistent with published data and with the results of this study, no significant difference was observed, as was the case for GCs in MCF-10A and MDA-MB-468 cells (Figure 3B,C). Increased expression of KLK10 upon DHT stimulation was observed in T-47D cells, as previously reported (Figure 3D) but no difference was observed for estradiol stimulation.

To study the kinetics of KLK10 protein expression after corticosteroid stimulation, a time-course experiment was performed. MCF-10A, MDA-MD-468 and T-47D cells were stimulated with different concentrations of betamethasone and the conditioned media were collected for KLK10 protein measurement on days 0, 1, 3 and 5 after hormonal stimulation. We found that KLK10 protein levels in the conditioned media started to increase 1 day after stimulation and continued to accumulate over the 5-day incubation time (Figure 4). KLK10 expression continued to increase in untreated MCF-10A cells up to day 5, while for cells treated with 10^{-6} M betamethasone, KLK10 expression was close to zero. For both MDA-MB-468 and T-47D cells, 10^{-6} M betamethasone-treated cells displayed higher KLK10 levels compared to control-treated cells. Thus, after day 1 of stimulation, the KLK10 expression levels were consistent with the above results regarding the expression differences for the three cell lines at the protein level.

To determine whether the GCs examined differentially regulate KLK10 production through their own receptors, we performed blocking experiments in which an antagonist, mifepristone (RU 486), was used to block their cognate receptors. Mifepristone is a widely used agent that blocks GRs and progestin receptors (Kacinski et al., 2001). It acts at the level of the receptor and competes with progesterone and cortisol for the corresponding binding sites in the ligand-binding domain (Baulieu, 1997). The aim of this experiment was to confirm that the

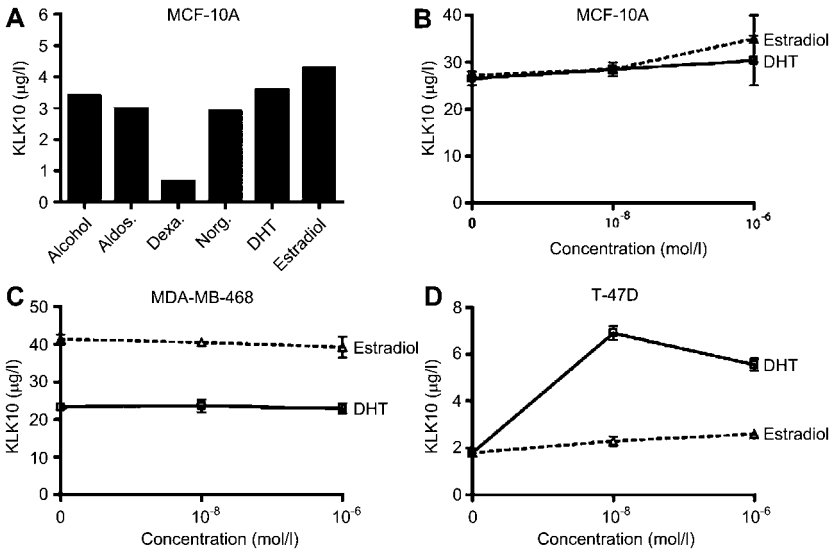

Figure 3 Stimulation of KLK10 by various hormones in breast cancer cell lines.
(A) MCF-10A cells stimulated with 10^{-8} M aldosterone (aldos), dexamethasone (dexa), norgestrel (norg), dihydrotestosterone (DHT) and estradiol for 7 days. (B) MCF-10A, (C) MDA-MB-468, and (D) T-47D cells stimulated with 10^{-8} and 10^{-6} M DHT and estradiol.

differential regulation of KLK10 is mediated by the cognate receptors. The cell lines were treated with the GR/PR antagonist RU 486 at a final concentration of 10^{-6} M, along with betamethasone, and incubated for 5 days in RPMI 1640 media containing charcoal/dextran-treated FBS. Figure 5 illustrates the effect of adding the antagonist RU 486 in the presence of betamethasone to the three cell lines on KLK10 expression (dotted lines). RU 486 reversed the effect of betamethasone alone in two of the three cell lines (MDA-MB-468 and T47-D). However, in MCF-10A cells, repression by the agonist (betamethasone) in the presence of RU 486 was not complete. This suggests that the mechanisms of induction and repression of KLK10 by GCs may be different in these three cell lines.

GR status in MCF-10A has not been deciphered, but the cell line is negative for estrogen receptor (ER) and PR (Lane et al., 1999; Neve et al., 2006). MDA-MB-468 cells display low levels of GR and are negative for ER and PR (Peterson and Barnes, 1991; Neve et al., 2006). T-47D cells have low levels of GR, high levels of ER and very high levels of PR (Konecny et al., 2003). To study the expression of GR on GC stimulation in these three cell lines, Western blot analysis was performed. Figure 6 illustrates the results of this study. It is clear that a basal level of GR expression exists in all three cell lines. While the levels of tubulin for the three cell lines are not the same, they are consistent and independent of GC treatment within each cell line. GR expression decreased in all three cell lines when stimulated with betamethasone. This is consistent with previous studies suggesting that most cell types down-regulate GR on GC exposure as part of a physiological feedback mechanism (Webster and Cidlowski, 1994). These data and those of Figure 5 suggest that the GR-mediated effects on KLK10 concentration after stimulation with GC may persist well beyond the GR down-regulation shown in Figure 6.

Currently, very little is known about the physiological function of KLK10 in breast tissue. In this study, we provided evidence of a differential expression pattern for KLK10 on GC stimulation in the three breast cell lines studied. The differential expression of KLK10 by various GCs is evident at both the mRNA and protein levels for two of the three cell lines, and the magnitude of the changes correlates well with established potencies of the hormones tested. Hormonal stimulation using other steroid hormones such as aldosterone, norgestrel, DHT, and estradiol did not yield significant differences in KLK10 expression in MCF-10A and MDA-MB-468 cells. Consistent with published results, only DHT stimulation of T-47D cells increased KLK10 expression. Blocking experiments using an antagonist of GR demonstrated that the observed effect was primarily due to GR-mediated effects for two of three cell lines (MDA-MB-468 and T47-D). The results suggest that the mechanisms of induction and repression of KLK10 by GCs in these cell lines may be different. Since RU 486 blocks both GR and PR, understanding the basis of the distinct physiology of GCs and progestins is limited by the paucity of genes and promoters that are differentially regulated by the two receptors (Wan and Nordeen, 2003). This is particularly important when considering the results for T-47D cells, since this cell line expresses low levels of GR but abundant levels of PR. For MCF-10A and MDA-MB-468, this is not a concern, as they have been shown to be negative for PR and the former has higher basal levels of GR compared to the other cell lines according to our Western blot data, while the latter has been shown to have low levels of GR (Neve et al., 2006). It is also possible that steroid hormone receptors are activated by non-steroidal ligands (Klocker et al., 1999). Western blot analysis of GR expression in these cell lines suggested that GR-mediated effects on KLK10 concentrations after stimulation with GC may persist well beyond the GR down-regulation. However, further studies are required to confirm these speculations. The *KLK10* promoter has been functionally and bioinformatically characterized to search for hormone response elements (HREs; Luo et al., 2003;

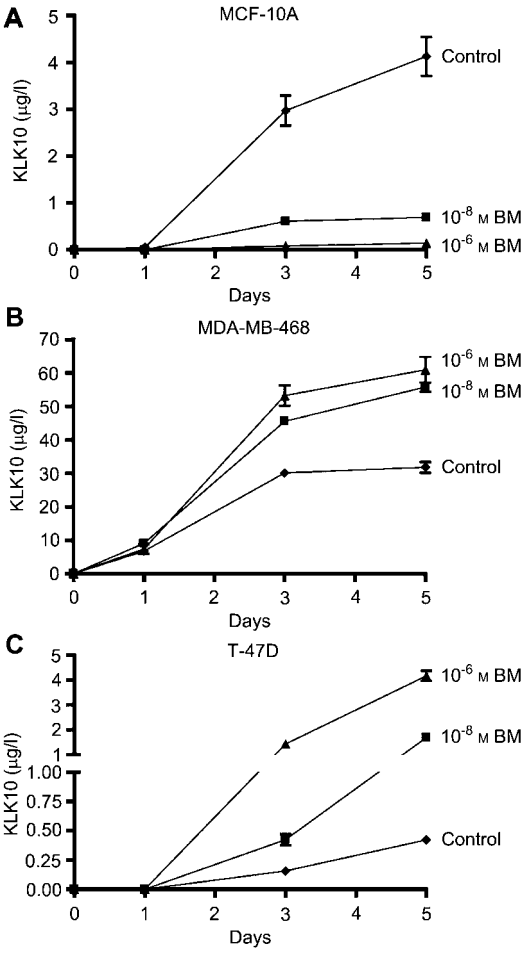

Figure 4 Kinetics of KLK10 protein production after induction with glucocorticoids.
MCF-10A, MDA-MB-468 and T-47D cells were cultured as described for the GC stimulation experiments. Conditioned media were collected for measurement of KLK10 protein expression (by ELISA) on days 0, 1, 3 and 5 after hormonal stimulation. Differential expression of KLK10 is observed in (A) MCF-10A, (B) MDA-MB-468 and (C) T-47D cells upon betamethasone stimulation, with MCF-10A displaying decreased KLK10 levels upon increased exposure to betamethasone and vice versa for the other two cell lines. Control, DMSO-treated cells; BM, betamethasone.

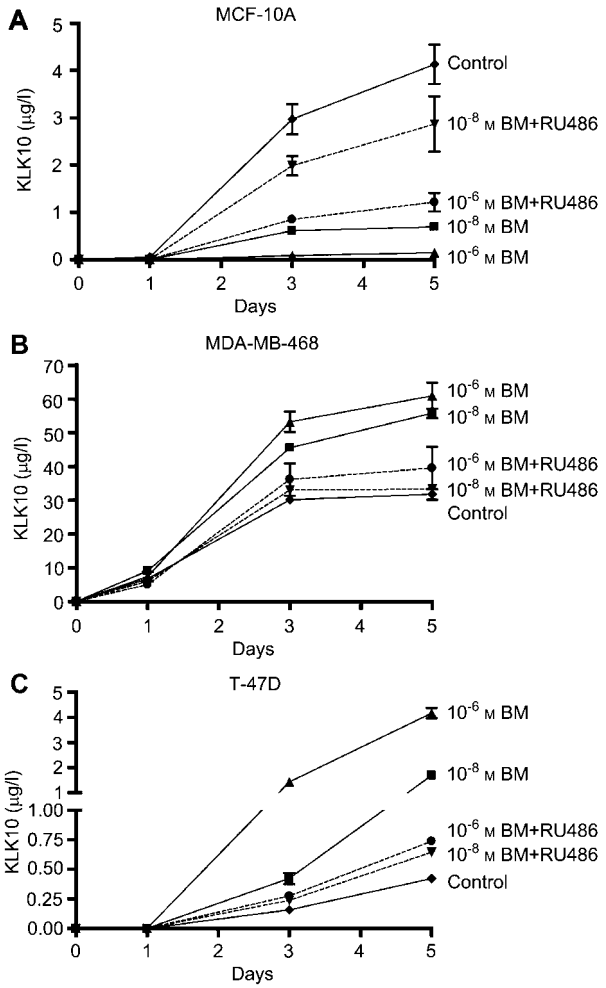

Figure 5 Blocking effect of the steroid hormone antagonist mifepristone on glucocorticoid-induced KLK10 protein production.
MCF-10A, MDA-MB-468 and T-47D cells were cultured as described in the GC stimulation experiments. An antagonist, RU 486 (mifepristone), a gift from Roussell UCLAF (Paris, France) was added to the culture media at a final concentration of 10^{-6} M and incubated for 5 days. Different betamethasone concentrations were concomitantly added to the culture media (10^{-6} and 10^{-8} M). The conditioned media were harvested for KLK10 measurement by ELISA at the end of the stimulation period. Addition of mifepristone (dotted lines) to MCF-10A (A), MDA-MB-468 (B) and T-47D (C) cells almost reversed the effects of betamethasone for two of the cell lines. Control, DMSO-treated cells; BM, betamethasone.

Paliouras and Diamandis, 2007). The authors did not identify any recognizable HREs, suggesting that coordinated binding of a number of coactivating factors along with the hormone receptor or a hormone-dependent activation via *trans*-acting factors might be required (Luo et al., 2003).

Previous results reported by Liu et al. (1996) suggest that many breast cancer cell lines, including T-47D (MDA-MB-468 was not examined), exhibit a significant decrease in KLK10 expression, while the normal mammary epithelial cells studied (MCF-10A was not examined) displayed abundant levels of KLK10. The basal level KLK10 in MDA-MB-468 cells is six-fold higher than the basal level in MCF-10A cells, which is contrary to the conclusion of Liu et al. that KLK10 is down-regulated in breast cancer cell lines.

Furthermore, CpG island hypermethylation of the *KLK10* gene has been suggested as being responsible for the loss of *KLK10* gene expression in breast cancer cell lines (Li et al., 2001). Specifically, there was a correlation between exon 3 hypermethylation and loss of *KLK10* mRNA expression in a variety of breast cancer cell lines (MDA-MB-468 and MCF-10A were not examined). T-47D was examined in this study; however, specific data were not reported for this cell line. Both MDA-MB-468 and T-47D cells were examined in another hypermethylation study and the results suggest that T-47D exhibits partial methylation status, while no data were presented for MDA-MB-468 (Sidiropoulos et al., 2005). These results suggest that *KLK10* is partially epigenetically suppressed or silenced through CpG island methylation. The current study proposes another mode of KLK10 regulation, namely by GCs via GR-dependent

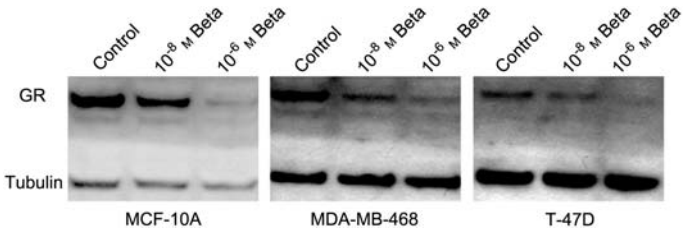

Figure 6 Correlation of glucocorticoid receptor expression with glucocorticoid concentration.
Western blot analysis shows that in cells treated with betamethasone (beta), increasing concentration of GC leads to decreased expression of GR, thus suggesting a possible physiological feedback mechanism. 250 000 cells were grown in 6-well plates for 48 h before they were treated with control (DMSO alone), or 10^{-6} or 10^{-8} M betamethasone and incubated for 5 days. Control and betamethasone-treated MCF-10A, MDA-MB-468 and T-47D cells were lysed using the CAT ELISA lysis buffer (Roche, Nutley, NJ, USA). The extracts were centrifuged and the supernatants stored at -80°C until further use. Protein concentrations were determined using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Samples of 5 μg of protein were boiled for 10 min and separated by 10% SDS-polyacrylamide gel electrophoresis. The gels were transferred to nitrocellulose membranes. For GR expression, the membrane was blocked in 5% bovine serum albumin (BSA) in Tris-buffered saline-Tween overnight at 4°C and probed with a primary mouse anti-GR monoclonal antibody (BD Biosciences Pharmingen, Rockville, MD, USA) at 1:200 for 1 h at room temperature in 1% BSA. Alkaline phosphatase goat-anti-mouse (H+L) was used as the secondary antibody at 1:5000 in 1% BSA (Jackson ImmunoResearch, West Grove, PA, USA). For tubulin expression, the membrane was blocked in 5% BSA for 1 h at room temperature and probed with a primary antibody (Cell Signaling Technology, Danvers, MA, USA) at 1:1000 dilution, overnight at 4°C in 1% BSA. Alkaline phosphatase goat-anti-rabbit (H+L) was used as the secondary antibody at 1:10 000 dilution in 1% BSA (Jackson ImmunoResearch). Specific immunoreactivity was demonstrated by adding chemiluminescent substrate for 10 min.

mechanisms. Given the recent implications of kallikreins in tumor progression and the numerous functions of GCs in physiological and pathophysiological conditions, it is crucial that further experiments be performed to examine in detail the mechanism by which GCs regulate KLK10 expression.

References

Adcock, I.M., Ito, K., and Barnes, P.J. (2004). Glucocorticoids: effects on gene transcription. Proc. Am. Thorac. Soc. *1*, 247–254.

Aranda, A. and Pascual, A. (2001). Nuclear hormone receptors and gene expression. Physiol. Rev. *81*, 1269–1304.

Baulieu, E.E. (1997). RU 486 (mifepristone). A short overview of its mechanisms of action and clinical uses at the end of 1996. Ann. NY Acad. Sci. *828*, 47–58.

Borgono, C.A. and Diamandis, E.P. (2004). The emerging roles of human tissue kallikreins in cancer. Nat. Rev. Cancer *4*, 876–890.

Cleutjens, C.B., Steketee, K., van Eekelen, C.C., van der Korput, J.A., Brinkmann, A.O., and Trapman, J. (1997). Both androgen receptor and glucocorticoid receptor are able to induce prostate-specific antigen expression, but differ in their growth-stimulating properties of LNCaP cells. Endocrinology *138*, 5293–5300.

Dhar, S., Bhargava, R., Yunes, M., Li, B., Goyal, J., Naber, S.P., Wazer, D.E., and Band, V. (2001). Analysis of normal epithelial cell specific-1 (NES1)/kallikrein 10 mRNA expression by *in situ* hybridization, a novel marker for breast cancer. Clin. Cancer Res. *7*, 3393–3398.

Fruchter, O., Kino, T., Zoumakis, E., Alesci, S., De Martino, M., Chrousos, G., and Hochberg, Z. (2005). The human glucocorticoid receptor (GR) isoform β differentially suppresses GRα-induced transactivation stimulated by synthetic glucocorticoids. J. Clin. Endocrinol. Metab. *90*, 3505–3509.

Goyal, J., Smith, K.M., Cowan, J.M., Wazer, D.E., Lee, S.W., and Band, V. (1998). The role for NES1 serine protease as a novel tumor suppressor. Cancer Res. *58*, 4782–4786.

Kacinski, B.M., Flick, M.B., and Sapi, E. (2001). RU-486 can abolish glucocorticoid-induced increases in CSF-1 receptor expression in primary human breast carcinoma specimens. J. Soc. Gynecol. Invest. *8*, 114–116.

Klocker, H., Culig, Z., Eder, I.E., Nessler-Menardi, C., Hobisch, A., Putz, T., Bartsch, G., Peterziel, H., and Cato, A.C. (1999). Mechanism of androgen receptor activation and possible implications for chemoprevention trials. Eur. Urol. *35*, 413–419.

Konecny, G., Pauletti, G., Pegram, M., Untch, M., Dandekar, S., Aguilar, Z., Wilson, C., Rong, H.M., Bauerfeind, I., Felber, M., et al. (2003). Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J. Natl. Cancer Inst. *95*, 142–153.

Lane, M.A., Romagnoli, L., Cruise, B., and Cohn, G.M. (1999). Spontaneous conversion to estrogen receptor expression by the human breast epithelial cell line, MCF-10A. Oncol. Rep. *6*, 507–511.

Leo, J.C., Guo, C., Woon, C.T., Aw, S.E., and Lin, V.C. (2004). Glucocorticoid and mineralocorticoid cross-talk with progesterone receptor to induce focal adhesion and growth inhibition in breast cancer cells. Endocrinology *145*, 1314–1321.

Li, B., Goyal, J., Dhar, S., Dimri, G., Evron, E., Sukumar, S., Wazer, D.E., and Band, V. (2001). CpG methylation as a basis for breast tumor-specific loss of NES1/kallikrein 10 expression. Cancer Res. *61*, 8014–8021.

Liu, X.L., Wazer, D.E., Watanabe, K., and Band, V. (1996). Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. Cancer Res. *56*, 3371–3379.

Lu, Y.S., Lien, H.C., Yeh, P.Y., Yeh, K.H., Kuo, M.L., Kuo, S.H., and Cheng, A.L. (2005). Effects of glucocorticoids on the growth and chemosensitivity of carcinoma cells are heterogeneous and require high concentration of functional glucocorticoid receptors. World J. Gastroenterol. *11*, 6373–6380.

Luo, L.Y., Grass, L., and Diamandis, E.P. (2000). The normal epithelial cell-specific 1 (NES1) gene is up-regulated by steroid hormones in the breast carcinoma cell line BT-474. Anticancer Res. *20*, 981–986.

Luo, L.Y., Grass, L., Howarth, D.J., Thibault, P., Ong, H., and Diamandis, E.P. (2001). Immunofluorometric assay of human kallikrein 10 and its identification in biological fluids and tissues. Clin. Chem. *47*, 237–246.

Luo, L.Y., Diamandis, E.P., Look, M.P., Soosaipillai, A.P., and Foekens, J.A. (2002). Higher expression of human kallikrein 10 in breast cancer tissue predicts tamoxifen resistance. Br. J. Cancer *86*, 1790–1796.

Luo, L.Y., Grass, L., and Diamandis, E.P. (2003). Steroid hormone regulation of the human kallikrein 10 (KLK10) gene in cancer

cell lines and functional characterization of the KLK10 gene promoter. Clin. Chim. Acta *337*, 115–126.

Neve, R.M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F.L., Fevr, T., Clark, L., Bayani, N., Coppe, J.P., Tong, F., et al. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell *10*, 515–527.

Novac, N. and Heinzel, T. (2004). Nuclear receptors: overview and classification. Curr. Drug Targets. Inflamm. Allergy *3*, 335–346.

Paliouras, M. and Diamandis, E.P. (2007). Coordinated steroid hormone-dependent and independent expression of multiple kallikreins in breast cancer cell lines. Breast Cancer Res. Treat. *102*, 7–18.

Peterson, G. and Barnes, S. (1991). Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. Biochem. Biophys. Res. Commun. *179*, 661–667.

Rittenhouse, H.G., Finlay, J.A., Mikolajczyk, S.D., and Partin, A.W. (1998). Human kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Crit. Rev. Clin. Lab. Sci. *35*, 275–368.

Sidiropoulos, M., Pampalakis, G., Sotiropoulou, G., Katsaros, D., and Diamandis, E.P. (2005). Downregulation of human kallikrein 10 (KLK10/NES1) by CpG island hypermethylation in breast, ovarian and prostate cancers. Tumour Biol. *26*, 324–336.

van Diest, P.J., van der Wall, E., and Baak, J.P. (2004). Prognostic value of proliferation in invasive breast cancer: a review. J. Clin. Pathol. *57*, 675–681.

Wan, Y. and Nordeen, S.K. (2003). Overlapping but distinct profiles of gene expression elicited by glucocorticoids and progestins. Recent Prog. Horm. Res. *58*, 199–226.

Webster, J.C. and Cidlowski, J.A. (1994). Downregulation of the glucocorticoid receptor. A mechanism for physiological adaptation to hormones. Ann. NY Acad. Sci. *746*, 216–220.

Yousef, G.M. and Diamandis, E.P. (2001). The new human tissue kallikrein gene family: structure, function, and association to disease. Endocr. Rev. *22*, 184–204.

Yousef, G.M., Yacoub, G.M., Polymeris, M.E., Popalis, C., Soosaipillai, A., and Diamandis, E.P. (2004). Kallikrein gene downregulation in breast cancer. Br. J. Cancer *90*, 167–172.

Zoorob, R.J. and Cender, D. (1998). A different look at corticosteroids. Am. Fam. Physician *58*, 443–450.

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