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13. Abstract ***(Maximum 200 Words)*** ***(abstract should contain no proprietary or confidential information)*** Most data suggest androgen/AR may be involved in proliferation of prostate cancer, opposite roles of androgen/AR in inhibition of cell growth and apoptosis are also documented. The detailed mechanism of how androgen/AR functions in apoptosis, however, remains unclear. A serine/threonine kinase (Akt) was demonstrated to play a role in promoting cell survival with anti-apoptotic effects. Akt was also found to be constitutively active in prostate cancer LNCaP cells and play an essential role for LNCaP survival. Our preliminary data demonstrated that Akt phosphorylates the androgen receptor (AR) at Ser210 and Ser790. A mutation at AR Ser210 results in reversion of Akt-mediated suppression of AR transactivation. Activation of phosphatidylinositol-3-OH kinase/Akt pathway results in the suppression of AR target genes. Our hypothesis is that Akt may control androgen/AR-induced apoptosis by phosphorylating and inhibiting AR. Our aims are to 1) prove that Akt can promote AR degradation via phosphorylation of AR *in vivo*, 2) dissect the molecular mechanism by which Akt promotes AR protein degradation, 3) determine whether Akt can suppress androgen/AR-induced cell growth inhibition and apoptosis, and 4) generate site-specific phospho-AR antibodies. Our project's success may enhance our understanding of cross-talk between Akt and androgen/AR pathway on prostate cancer progression.

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Table of Contents

INTRODUCTION:

Most data suggest androgen/AR may be involved in proliferation of prostate cancer, opposite roles of androgen/AR in inhibition of cell growth and apoptosis are also documented. The detailed mechanism of how androgen/AR functions in apoptosis, however, remains unclear. A serine/threonine kinase (Akt) was demonstrated to play a role in promoting cell survival with anti-apoptotic effects. Akt was also found to be constitutively active in prostate cancer LNCaP cells and play an essential role for LNCaP survival. Our hypothesis is that Akt may control androgen/AR-induced apoptosis by phosphorylating and inhibiting AR. Our aims are 1) to prove that Akt can promote AR degradation via phosphorylation of AR *in vivo*, 2) to dissect the molecular mechanism by which Akt promotes AR protein degradation, 3) to determine whether Akt can suppress androgen/AR-induced cell growth inhibition and apoptosis, and 4) to generate site-specific phospho-AR antibodies and use these Abs to monitor the AR phosphorylation status and their relationship to the progression of prostate cancer in archival human tissues. Our project's success may enhance our understanding of cross-talk between Akt and androgen/AR pathway on prostate cancer progression.

BODY:

Aims 1: To prove that Akt can promote AR degradation via phosphorylation of AR *in vivo*. Our preliminary studies indicated that Akt phosphorylates AR *in vitro* and could suppress AR transactivation in prostate cancer cells through the Akt upstream activator phospho-inositol 3 kinase (PI3K). In order to further prove this phenomenon, we applied the PI3K inhibitor LY294002 in LNCaP cells to block the PI3K/Akt pathway, to wee whether AR expression and activity can be really influenced by this signaling pathway. We treated LNCaP cells with LY294002 for 24 hr (*in vivo* tissue culture assay), then performed the Western blot assay. Several experiments were performed with various passage numbers of LNCaP cells and the results are represented in Fig. 1. LY294002 treatment increased AR and the phosphate specific antigen (PSA) protein level. This indicates that the blockade of this PI3K/Akt pathway causes increased AR expression and activity, proving the *in vivo* phosphorylation of AR by Akt through the PI3K pathway. Additional studies are ongoing.

Aim 2: To dissect the molecular mechanism by which Akt promotes AR protein degradation. The same *in vivo* studies described in Aim 1 above are also mechanistic studies. The LY294002 treatment assays were carried out for various time points and demonstrated that AR activity was not induced within 4 hours but after 24 hours there was a strong enhancement of AR activity, as shown in Fig. 2. This suggests that this is a genomic effect. In order to further prove that LY294002 treatment increases AR expression and the activity is an AR-dependent phenomenon, we used AR-RNAi to impair AR expression. RNAi is a specially designed double-stranded RNA, which can degrade targeted RNA. As shown in Fig. 3, (using the assays described in Aim 1 above) RNAi reduced the LY294002 inducing effect on AR and PSA protein expression (lane 8 versus 6), indicating that LY294002 effect is AR-dependent. Several other molecular mechanism studies are planned for the coming year.

Aim 3: To determine whether Akt can suppress androgen/AR-induced cell growth inhibition and apoptosis. Studies for this aim are planned for the coming year.

Aim 4: To generate site-specific phospho-AR antibodies and use these Abs to monitor the AR phosphorylation status and their relationship to the progression of prostate cancer.
Several site-specific phospho-AR antibodies have been recently developed. They are presently in the process of being tested for their specificity and to determine appropriate and efficient testing concentrations and procedures. The studies on archival human tissue samples will proceed after the evaluations of the antibodies are completed.

KEY RESEARCH ACCOMPLISHMENTS:

- Akt phosphorylates AR and suppresses AR transactivation in prostate cancer cells *in vivo.*
- The blockage of the PI3K/Akt pathway is AR-dependent and genomic.
- Site-specific antibodies developed and under evaluation.

REPORTABLE OUTCOMES:

- One manuscript associated with the proposal submitted to Journal of Biological Chemistry.

CONCLUSIONS:

As a summary, we ask how to interpret these findings and what is the physiological role of increased AR function after PI3K/Akt is blocked? We found that removal of androgens in LNCaP cells resulted in increased levels of active phosphorylated Akt. Thus, we believe that the AR and PI3K/Akt signaling both appear to be important proliferation and survival factors in prostate cancer cells, and seem to antagonize each other to maintain the cell homeostasis. The AR activity can be induced by LY294002 to lay a dominant proliferation role to compensate for the loss of PI3K/Akt signaling.

REFERENCES: None

APPENDICES: Figures 1, 2, and 3

Fig. 1. Inhibition of PI3K/Akt signaling enhanced AR and PSA protein expression level. The LNCaP cells (passage number of P23 and P61) were cultured in 10% Charcoal Dextran treated FBS-medium for 24 hr and then treated with vehicle, DHT, or DHT plus 20 mM LY294002. After 24 hr, cells were harvested to do Western-blot analysis with AR, PSA, and b-actin antibodies as indicated.

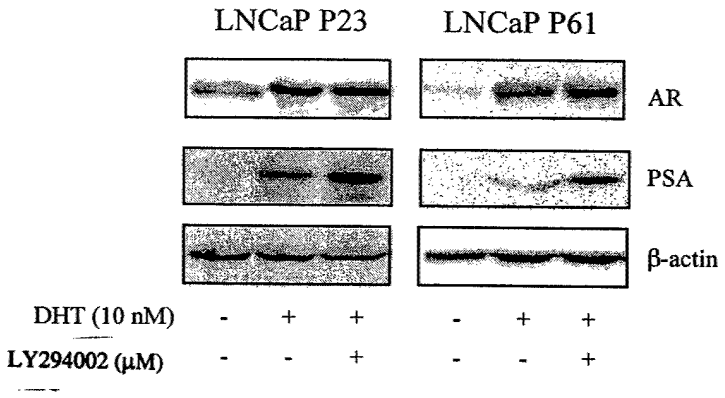

Fig. 1 Blockade of PI3k/Akt pathway causes increased AR expression and activity.

Fig. 2. LY294002 treatment increased AR activity is time-dependent. The LNCaP cells (passage number P23) were cultured in 10% Charcoal Dextran treated FBS-medium for 24 hr and then treated with vehicle, DHT, or DHT plus 20 mM LY294002. After 4 hr and 24 hr, cells were harvested to do western-blot analysis with PSA and b-actin antibodies as indicated.

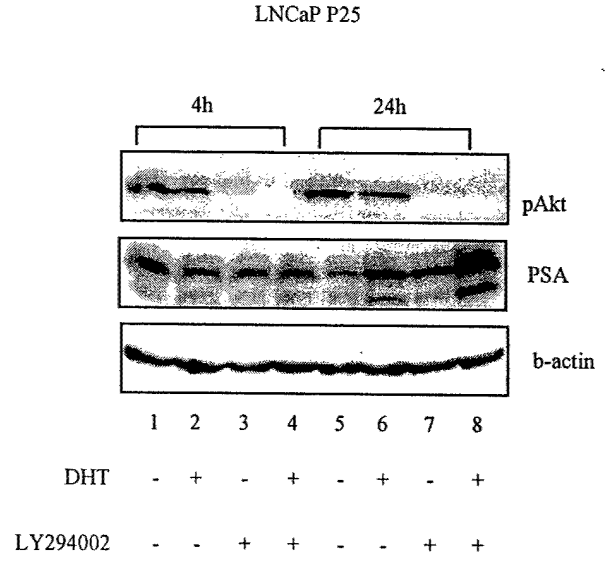

Fig. 2 LY294002 enhanced AR activity is time-dependent.

Fig. 3. AR-RNAi reduced AR and PSA expression. The LNCaP cells (passage number P23) were cultured in 10% Charcoal Dextran treated FBS-medium for 48 hr, then transfected with AR-RNAi for 22 hr, followed by treatment with ethanol, 10 nM DHT, or combined with 20 mM LY294002 for another 24 hr. The cell were harvested to do western-blot analysis with AR, PSA, and b-actin antibodies as indicated.

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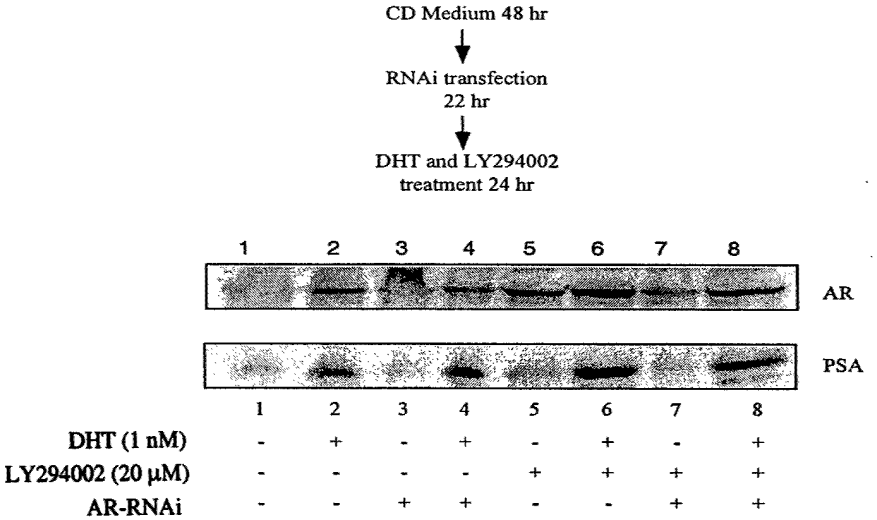

(Loading amount has been normalized to 80 μg/lane)

Fig. 3 LY2942002 increased AR activity is AR-dependent