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Award Number: W81XWH-08-1-0060

TITLE: Control of Akt Activity in Prostate Cancer

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REPORT DATE: February 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE 06/02/2009		2. REPORT TYPE Annual		3. DATES COVERED 7 JAN 2008 - 6 JAN 2009	
4. TITLE AND SUBTITLE Control of Akt Activity in Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-08-1-0060	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Albert S. Baldwin, Ph.D. Email: albert_baldwin@med.unc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of North Carolina at Chapel Hill Chapel Hill, NC 27599				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) US Army Medical Research and Materiel Command Fort Detrick, MD 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S) NUMBER(S)	
				11. SPONSOR/MONITOR'S REPORT	
12. DISTRIBUTION / AVAILABILITY STATEMENT Aporoved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Akt is an important kinase in prostate cancer, being activated downstream of growth factor receptors and downstream of PTEN mutations. Akt activity is controlled through two mechanisms, phosphorylation by Pdk1 and by Pdk2. Pdk2 is the mTORC2 complex, which controls phosphorylation of Akt at Ser473. We have recently shown that IKK α , a component of the IKK complex which controls NF- κ B activation, regulates mTORC1 downstream of Akt activation. Here we explore a role for IKK α in controlling mTORC2 activity. Our data indicate that IKK α associates with mTORC2 in prostate cancer cells and regulates its activity towards Akt at Ser473. Knockdown of IKK α blocks Akt activity in prostate cancer cells. We propose to determine the mechanisms whereby IKK α controls mTORC2 activity and to determine if loss of IKK α in a animal model for prostate cancer will block progression of the disease. We will test an IKK α inhibitor as a potential therapeutic for prostate cancer in this model.					
15. SUBJECT TERMS Prostate cancer, Akt activity, IkappaB kinase, mTOR, animal model					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

Our goal is to understand how IKK α (a key component of the IKK complex that controls NF- κ B activation, see Basseres and Baldwin, 2006) controls Akt in prostate cancer cells, and to determine if IKK α controls development of prostate cancer in an animal model. NF- κ B has been reported to be important for prostate cancer progression (Domingo-Domenech et al., 2005). Akt is the key effector in the PI3K pathway and Akt is important in controlling prostate cancer development (Chen et al., 2006). Loss of the tumor suppressor PTEN is sufficient to lead to the development of prostate cancer in an animal model (Wang et al., 2003) and loss of PTEN leads to constitutively active Akt (Hay 2005). The work described here is part of our ongoing effort to understand the interrelationship between the IKK signaling pathway and both mTOR signaling components (mTORC1 and mTORC2) (see Kim et al., 2002; Bjornsti and Houghton, 2004; Dutcher 2004; Dan et al., 2007). Our preliminary data indicated that IKK α associates with the mTORC2 complex in prostate cancer cancers. mTORC2 is the PDK2 activity that phosphorylates Akt at ser473 to control its activity (Sarbasov et al., 2005). Our data show that knockdown of IKK α in prostate cancer cells blocks phosphorylation of Akt at ser473, the known target sequence for mTORC2. Ultimately we want to analyze the role of IKK α in controlling prostate tumorigenesis in an animal model, and to bring

BODY:

Regarding the original Aim 1 goals, we have:

--(Aim 1.1). We have assayed a number of cancer cells to determine whether IKK α controls Akt S473 phosphorylation. In most cancer cells, we find that knockdown of IKK α regulates Akt S473 phosphorylation. **Fig. 1** shows the results of knockdown of IKK α relative to the regulation of Akt phosphorylation in two prostate cancer cells. In these cells, IKK α associates with mTORC2 as shown by co-immunoprecipitation studies (**Fig. 2**). In these studies, immunoprecipitation of IKK α pulled down both Raptor and Rictor, consistent with the association with mTORC1 (Dan et al., 2007) and with mTORC2 (**Fig. 2, left panel**). Immunoprecipitation of Rictor (defining mTORC2) only pulled down mTOR and IKK α and not Raptor (which defines mTORC1) (**Fig. 2, right panel**). In one breast cancer cell we have found that knockdown of IKK α in those cells did not block Akt phosphorylation at ser473. We utilized the PI3K inhibitory LY and found that it did not block association of IKK α with mTORC2 in PC3 cells, indicating that Akt does not drive an association between IKK α and mTORC2 (data not shown).

--(Aim 1.3). We assayed Pdk1 activity following knockdown of IKK α and found that its kinase activity is not diminished (**Fig. 4**). Thus, this data indicates that the effect of IKK α on Akt activity is through mTORC2 and not through Pdk1 (data not shown).

--(Aim 1.4). We have asked whether loss of IKK α will block the upregulation of Akt ser473 phosphorylation found when mTORC1 is inhibited. The upregulation of Akt activity is seen when cancer cells are treated with rapamycin, which blocks mTORC1 and inhibits S6K-phosphorylation. This then blocks the ability of S6K to inhibit IRS-1, a downstream mediator of growth factor-induced Akt activation, allowing growth factor-induced upregulation of Akt (O'Reilly et al., 2006). Our data indicate that loss of IKK α blocks the upregulation of Akt S473 phosphorylation when Raptor (a critical component of mTORC1) is knocked down (**Fig. 3**). This result has important implications for cancer therapy.

Regarding Aim 2 goals, we have:

--(Aim 2.2). We have crossed the IKK α fl/fl animal with the PB-Cre animal. These animals are now being crossed with the PTEN fl/fl animal. Once we have enough founder animals we can begin our studies to determine if IKK α is required for development of prostate cancer downstream of loss of PTEN.

KEY RESEARCH ACCOMPLISHMENTS:

--Clear demonstration that in all prostate cancer cell lines tested (those that are PTEN+ or PTEN-) that IKK α controls Akt activity in these cells.

--Loss of IKK α controls Akt-induced phosphorylation of Foxo3a but not TSC2 (consistent with knockdown of Rictor).

--IKK α is associated with mTORC2 in several cell types, including prostate cancer cells.

--IKK α does not control Pdk1 activity (which controls Thr308 phosphorylation of Akt).

--Loss of IKK α blocks induced Akt activity associated with inhibition of mTORC1, indicating the potential of IKK α inhibition as a mechanism to block both mTORC1 (previous study) and mTORC2, providing a significant advantage of rapamycin treatment.

--Successful cross of IKK α fl/fl animal with PB-Cre animal, in preparation for animal tumor studies.

REPORTABLE OUTCOMES:

--Manuscript is in preparation describing these findings.

--New funding (NIH grant awarded based on these studies)

CONCLUSION:

Our results clearly demonstrate that IKK α is a key regulator of Akt activity in prostate cancer cells (and potentially other cancer cells). Since Akt is a key regulator of oncogenesis in prostate cancer, our work opens the potential that an IKK α inhibitor could be therapeutic in prostate cancer. Our animal model, which is being developed, will provide key genetic evidence for this hypothesis.

REFERENCES:

- Basseres, D. and Baldwin, A. NF- κ B and IKK pathways in oncogenic initiation and progression. *Oncogene* 25, 6817-6830, 2006.
- Bjornsti, M.A. and Houghton, P.J. The mTOR pathway: a target for cancer therapy. *Nat. Rev. Cancer* 4, 335-348, 2004.
- Chen, M.L., Xu PZ, Peng XD, Chen WS, Guzman G, Yang X, Cristofano A, Pandolfi PP, and Hay N. 2006. Deficiency of Akt is sufficient to suppress tumor development in PTEN +/- mice. *Genes and Dev.* 20, 1569-1574.
- Dan, H., Adli, M., and Baldwin, A. IKK α controls mTOR activity in PTEN-inactive prostate cancer cells. 2007. *Cancer Res.* 67, 6263-6269.
- Domingo-Domenech, J., Mellado, B., Ferrer, B., Truan, D., Codony-Servat, J., Sauleda, S., Alcover, J., Campo E., Gascon, P., Rovira, A., Ross, J.S., and Albanell, J. 2005. Activation of NF- κ B in human prostate carcinogenesis and association to biochemical relapse. *Br. J. Cancer* 93, 1285-1294.
- Dutcher, J. P. Mammalian target of rapamycin inhibition. *Clin. Cancer Res.* 20, 6382-6387, 2004.
- Hay, N. 2005. The Akt-mTOR tango and its relevance to cancer. *Cancer Cell* 8, 179-183.
- Kim, D.-H., Sarbassov, D., Ali, S., King, J., Latek, R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. 2002. mTOR interacts with Raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell* 110, 163-175.
- O'Reilly, K., Rojo, F., She, Q., Solit, D., Mills, G.B., Smith, D., Lane, H., Hofmann, F., Hicklin, D., Ludwig, D., Baselga, J., and Rosen, N. mTOR inhibition induces upstream tyrosine kinase signaling and activates Akt. *Cancer Res.* 66, 1500-1508, 2006.
- Sarbassov, D., Guertin, D., Ali, S., and Sabatini, D. 2005. Phosphorylation and regulation of Akt/PKB by the Rictor-mTOR complex. *Science* 307, 1098-1101.
- Wang, S., Gao, J., Lei, Q., Rozengurt, N., Pritchard, C., Jiao, J., Thomas, G., Li, G., Roy-Burman, P., Nelson, P., Liu, X., and Wu, H. 2003. Prostate-specific deletion of the murine PTEN tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* 4, 209-221.

APPENDIX

FIGURE LEGENDS

Figure 1. IKK α positively regulates both Akt and mTOR phosphorylation. (A) PC3 and LNCaP cells were transfected with control siRNA, and siRNAs to IKK α and IKK β as indicated. The cells were lysed 48 hrs after transfection and the levels of IKK α , IKK β , and β -tubulin and endogenous phosphorylation of S6K and Akt were determined by immunoblotting with the indicated antibodies. The experiment shown is representative of three different experiments.

Figure 2. IKK α associates with both mTORC1 and mTORC2. (A) and (B) Immunoprecipitations of endogenous IKK α (A) or Rictor (B) prepared from PC3 cells were analyzed for endogenous IKK α , mTOR, Rictor or Raptor levels using the indicated antibodies. Mouse (A) or rabbit IgG were used as negative control, and total cell lysate (1% input) indicates expression of mTOR complex.

Figure 3. Loss of IKK α suppresses the loss of feedback control on Akt when mTORC1 is inhibited. PC3 cells were transfected with siRNA IKK α or with siRNA to Raptor. Knockdown of Raptor suppresses mTORC1 activity. The levels of endogenous phosphorylation of S6K, IRS-1, and Akt as well as of IKK α , mTOR and β -tubulin were determined by immunoblotting with the indicated antibodies. Results are representative of three experiments.

Figure 4. Knockdown of IKK α does not block activation status of PDK1. Extracts were prepared from PC3 cells knocked down for IKK α which were analyzed with an antibody specific for phospho-S241 on PDK1 (which is reflective of the activity of PDK1).

FIGURES

Figure 1

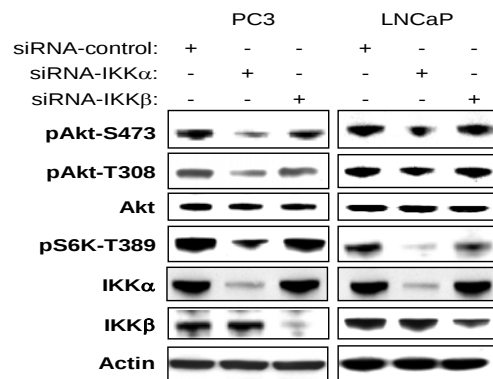


Figure 2

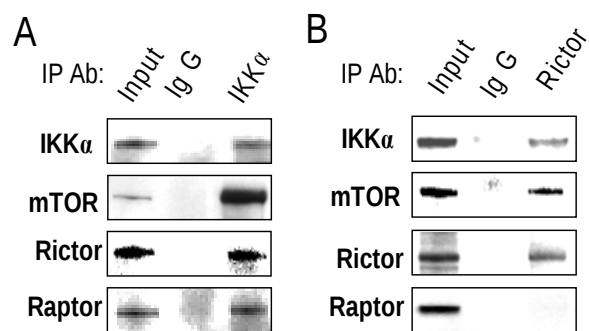


Figure 3

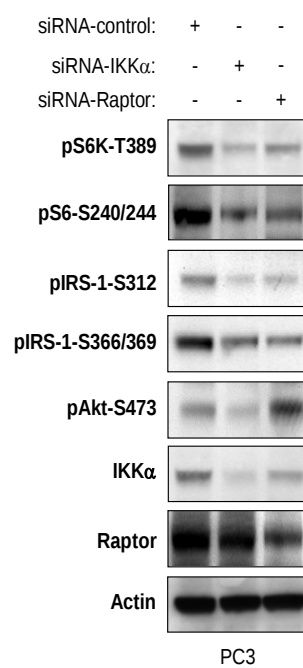


Figure 4.

