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In the current funding year, we focus our effort on proposed work for Tasks 2 and 3 to characterize both the functional and physiological roles of caveolin in AR signaling. We have demonstrated a corss-talk between the caveolin-1/AR and PI3 kinase/Akt signal pathway in hormone dependent cell survival. Our results established a biochemical basis on the notion that caveolin expression is associated with prostate cancer progression. The "neo-expression" caveolin in advanced prostate cancer represents a gain-of-function event in cancer progression. Moreover, these results illustrate the important role of AR non-genomic effect in response to androgen stimulation. These findings pave the way to further define the underlying signal cross-talk in AR-mediated signaling.

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DOD Progress Report:

Caveolin-1 Modulates Androgen Receptor Signaling in Advanced Prostate Cancer

Introduction:

The underlying mechanism of the progression of prostate cancer to hormone-independent disease is poorly understood. Neoexpression of caveolin-1, a scaffold protein associated with caveolae membrane microdomains, has been shown to correlate with hormone resistance and metastasis in both human and mouse prostate cancer models (Nasu et al., 1998; Yang et al., 1999). We find that overexpressing caveolin-1 in human prostate cancer cells positively regulates androgen receptor transactivation activity. We identify that modulating caveolin expressing levels dramatically alter the sensitivity of AR to androgen stimulation in cellular models (Lu et al., 2002). We hypothesize that caveolin-1 scaffolding signal complex plays a regulatory role in AR activation pathway. Our specific aims are: (1) Mapping the submolecular domains required for AR and caveolin interaction; (2) Functional and biochemical characterization of the AR/caveolin interaction; (3) Characterization of the physiological role of caveolin-1 overexpression in AR signaling of prostate carcinoma cell; (4) Evaluating the effect of caveolin scaffolding domain CSD peptide in prostate cancer PC3 tumor growth in vivo. The immediate goal of the present proposal aims to identify and characterize the sub-molecular domains involved in AR/caveolin interaction.

Report Body:

Overview:

In the current funding year, we focus our effort on proposed work for Tasks 2 and 4 characterizing both the functional and physiological roles of caveolin in AR signaling. Using a mammalian two-hybrid assay, we determined two caveolin interacting domains in androgen receptor (AR) which localized to AR N-terminus and ligand binding domain (LBD). The AR binding domain of caveolin was determined to localize to the region (Cav30-60) preceding the caveolin scaffolding domain (CSD) of caveolin (Lu et al., 2001). These interactions were verified biochemically by GST pull-down and co-immunoprecipitation.

1. Identification of inhibitory caveolin peptide in perturbing AR/caveolin interaction:

Various caveolin domains, including CSD (caveolin scaffolding domain), Cav(1-30), Cav(1-60), Cav(31-60) and Cav(135-178), were cloned into mammalian pcDNA3.0 expression vector. Their effects on interfering with AR/caveolin interaction were tested by a co-transfection study using a previously defined pACT-AR/pBIND-CAV9FL) mammalian two-hybrid assay. As shown in Figure 1, among peptides tested, peptides Cav(31-60) and Cav(135-178) appeared to be most effective in perturbing the caveolin/AR interaction. This is consistent with our previous results which identified Cav(31-60) as an AR interacting domain.

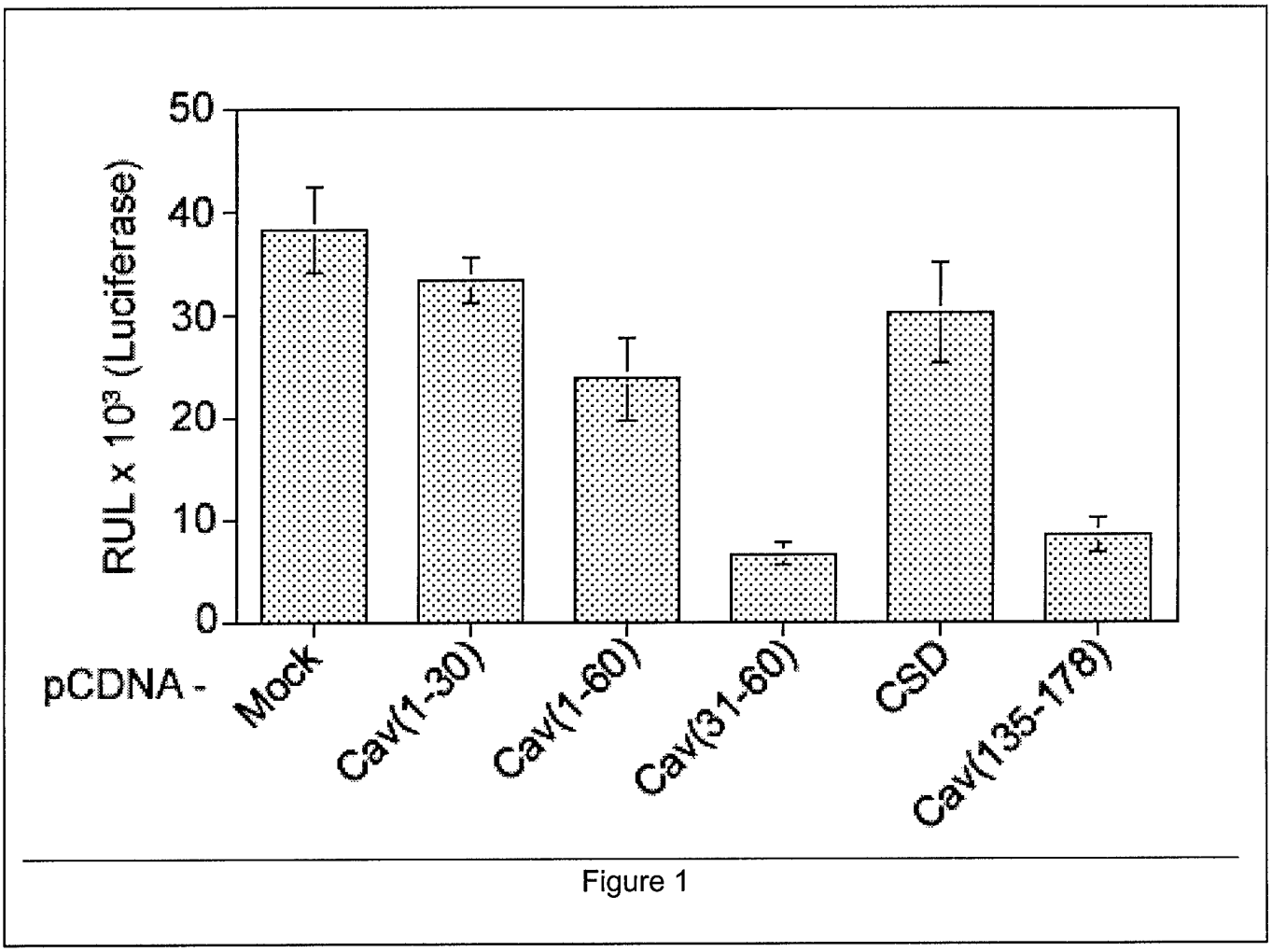

Figure 1

2. Cav(137-178) downregulatesPI3k/Akt signal in LNCap cells.

To test the effects of caveolin peptides in modulating androgen-mediated signals, LNCap cells were transiently transfected with various expression vectors encoding the caveolin peptides. 24 hours post-transfection, the level of androgen-stimulated Akt activation was determined by western blot analysis using a Ser-473 phospho-specific antibody (Li et al., 2003). As shown in Figure 2, an increase of Akt Ser-473 phosphorylation was observed in LNCap cells in response to androgen

stimulation. While transient transfection with vectors harboring Cav(1-30), Cav(30-60) or CSD does not alter the androgen-induced Akt activation, expression of the Cav(135-178) peptide dramatically downregulates the androgen-stimulated Akt activation since Cav(135-178) was mapped to be an AR interacting domain. Our data suggest the possibility that down-regulation of androgen stimulation-induced Akt activation may be caused by disrupting caveolin/AR interaction.

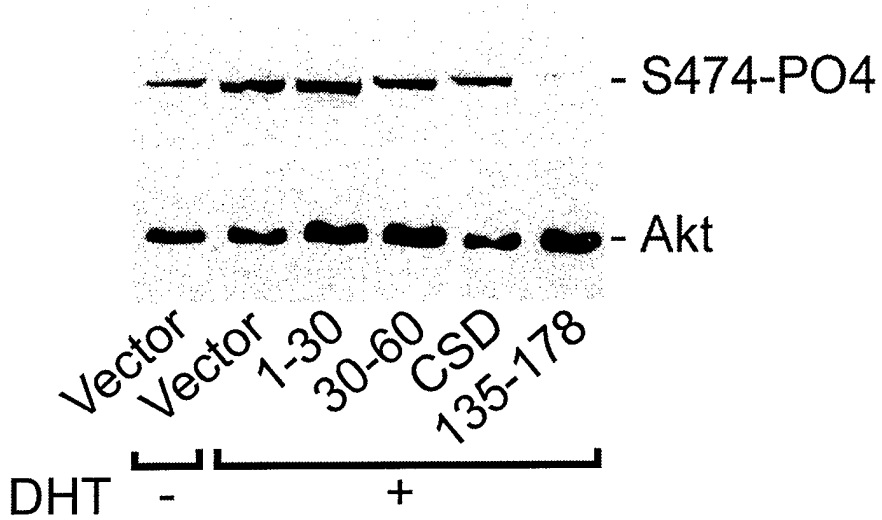

Figure 2

3. Testing caveolin specific siRNA to knock-down caveolin expression in PC3 prostate cancer cells.

To further demonstrate the role of caveolin in promoting prostate cancer cell survival, we have initially characterized a pair of caveolin RNAi oligonucleotides for their ability to downregulate caveolin expression in PC3 cells (Paddison et al., 2002) (gene bank accession number AF070648):
#139-GGUCAGCAGCCUCCCUGAA
#256-CAUCUUUAUCCGUAGUGGG.
As shown in Figure 3, PC3 cells were transiently transfected with 10 nM of scrambled control (SC), #139-, or #256- siRNAs usning lipofectamine 2000. 72 hours post-transfection, levels of caveolin expression are determined by a western blot analysis . As shown in Figure 3, a dramatic decrease of caveolin protein level is observed in caveolin specific siRNA #139 and #256. Preliminary results indicate that caveolin knock-down induces apoptosis in these cells (data not shown).

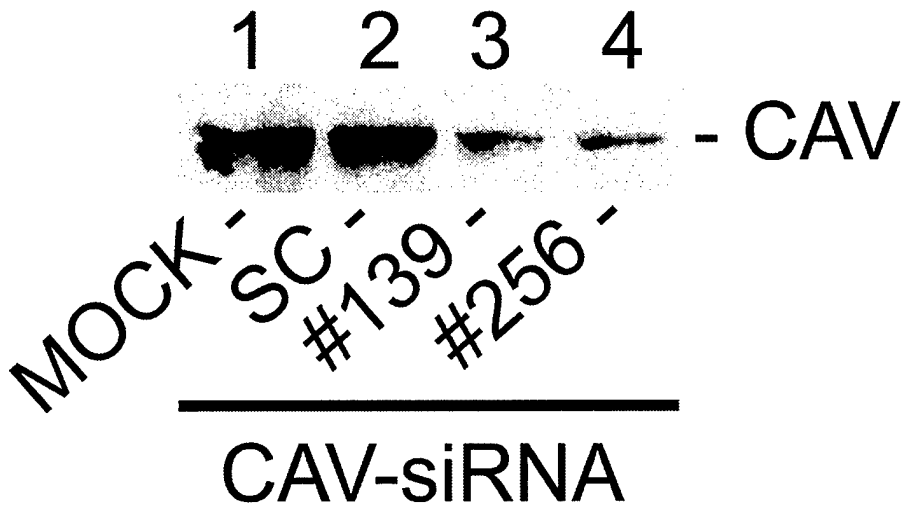

Figure 3

Future direction and Plan:
No change in research direction is anticipated for the next project year. The main goal for the coming year will be to test the efficacy of caveolin small interference RNA to further substantiate our model. Once the RNAi method is established, a viral vector system will be employed. We anticipate the knock-down approach will demonstrate unequivocally that caveolin neoexpression is a gain of function event in tumor progression. Once these tools are fully characterized, we will test both small peptide and the small interfering RNA (siRNA) in an animal model as described in Aim 4.

Key Research Accomplishments:

1. Determine the biological effects of various caveolin fragments on AR mediated signaling.

2. Established the physiological interactions between AR and caveolin-1 at both molecular and biochemical levels.

3. Characterized the feasibility of using small interference RNA (siRNA) in caveolin expression knock-down study in tissue culture model.

Reportable Outcomes:

1. Manuscript:
   Zhang A., Liu X., Liao G. and Lu M.L. Neo-expression of Caveolin-1 Promotes Cell Motility and Survival in Prostate Cancer Cells. (Manuscript in preparation.)
2 Human caveolin 1 specific Small interference RNAs.
3. Expression Vectors:
   Mammalian expression vectors for AR, caveolin expression. pcDNA-AR. pcDNA-Cav(1-30), Cav(31-60), Cav(137-178)

Conclusion:

In summary, we have demonstrated a cross-talk between the caveolin-1/AR and PI3 kinase/Akt signal pathway in hormone dependent cell survival. Overall, our results established a biochemical basis on the notion that caveolin expression is associated with prostate cancer progression. The "neoexpression" caveolin in prostate cancer progression represents a gain of function event in cancer survival. Moreover, these results illustrate the important role of AR non-genomic effect in response to androgen stimulation. These findings pave the way to further define the underlying signal cross-talk in AR-mediated signaling.

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