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TITLE: Castration Induced Neuroendocrine Mediated Progression of Prostate Cancer

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unless so designated by other documentation.
In the past twelve months we have demonstrated that bombesin stimulates the androgen receptor preferentially to a proximal androgen response element in the promoter region rather than in the enhancer region, which is primarily stimulated by androgens. We have shown that gastrin-releasing peptide prostate cancer cells have their growth in soft agar inhibited by the specific Src inhibitor AZD0530. This is a dose-dependent response. AZD0530 abolishes the nuclear translocation of the androgen receptor demonstrating specificity. We have also demonstrated that AZD0530 inhibits metastases of gastrin-releasing peptide prostate cancer cells in a SCID mouse model. Finally, we have delineated the downstream signaling cascades of neuroendocrine activation of androgen independent prostate cancer cell growth and have effectively inhibited these using the novel Src kinase inhibitor.
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**Introduction**

We believe that androgen withdrawal is an event that initiates a cascade promoting the development of androgen independence through NE progression. To date we know of no adjuvant therapies targeting castration initiated molecular events in clinical practice. As such, we seek to better define these early post-castration molecular events. We hypothesize that a small population of neuropeptide expressing AI CaP cells generated by castration can support the AI survival and growth of androgen sensitive cells in a paracrine fashion. This concept is a novel one regarding the early propagation of CaP following castration. Secondly, we hypothesize that neuropeptide mediated non-receptor tyrosine-kinase signaling activates androgen regulated genes both through AR and GRP dependent, and AR and GRP independent mechanisms. Demonstration of this concept establishes the rationale for neuropeptide pathway inhibition as singular and combination therapy at the time of castration.

**Body**

**Aim 1.** To determine the paracrine effect of NE cells on androgen sensitive CaP cells.

a. *Determine the in vitro ability for NE cells to support androgen sensitive CaP cell survival and growth (paracrine effect) in androgen-deprived conditions.* Work on this section was replaced by the soft agar assay as results in soft agar are more definitive.

b. *Determine the paracrine effect in soft agar tumorigenesis.* Work on this section is concluded as reported in the 2006 annual report.

c. *Determine the paracrine effect on migration in recombinant NE cells.* Work on this section is concluded as reported in the 2006 annual report.

d. *Study the paracrine effect using the in vivo xenograft model with regard to growth and metastasis.* Work on this section is concluded as reported in the 2006 annual report.

**Aim 2.** To evaluate the mechanisms of AR involvement in our NE model.

a. *Testing of inhibition of neuropeptides, signaling molecules and AR inhibitors individually and in combination on soft agar growth of GRP clones and xenograft cells.* The mechanisms of neuropeptide-mediated AR activation were investigated in more details this year. We performed chromatin immunoprecipitation (ChIP) assay and discovered that bombesin-stimulated AR binds preferentially to the proximal ARE site in the promoter region rather than the enhancer region bound by the androgen-stimulated AR. GRP-Pro cells constitutively expressing GRP have the AR occupied on the proximal ARE constantly. This bombesin/GRP-stimulated preferential binding of AR to the proximal site of the PSA promoter is assisted by the AR co-activator ACTR 30 min from addition of bombesin.
As reported last year, growth of GRP cells in soft agar may be inhibited by the specific Src inhibitor AZD0530. We performed a dose-response growth inhibition curve using GRP-Pro cells grown in CS media and treated with various doses of AZD0530. The IC50 for this inhibition is slightly higher than 1 µM. The LNCaP GRP cell lines have demonstrated promoted migratory activities than their parental cells. Src kinase inhibitor AZD0530 inhibits the migration assayed by the Boyden chamber assay to the levels similar to the basal activity in the LNCaP cells.

LNCaP GRP cells showed translocalization of AR into the nuclei in the absence of androgen stimulation (in CS growth media) compared to the mock-transfected LNCaP Zeo cells. Addition of Src kinase inhibitor AZD0530 abolished the AR nuclear translocalization as shown in the left. This result suggests that AR is activated through autocrine stimulation of GRP that is dependent of Src
We surveyed the status of Src and FAK in the LNCaP and GRP subclones and found similar levels of phosphorylated Src and FAK kinases. However, when these two kinases were co-immunoprecipitated by anti-FAK antibodies, stronger phospho-Src levels were detected in GRP subclones than their mock control Zeo cells. These findings confirm our hypothesis that in the absence of AR, bombesin/GRP bind to their receptors, activate Src and FAK kinases in the complex and activate AR through phosphorylation.

b. Small hairpin RNA (shRNA)-based silencing of NE cells in vitro and in vivo. We are in the process of designing the shRNA. Once we get the shRNA construct, we will start experiments in this section.

c. Testing of inhibitory treatments on chimeric tumors in soft agar and in vivo. We have demonstrated inhibition of paracrine migration. We are presently testing inhibition of chimeric tumor growth and metastasis in vivo.

d. In vivo testing of inhibitory treatments at different time points. Since we have identified Src kinase as the key player in neuropeptide-mediated AR activation, we tested the effect of Src kinase inhibitor AZD0530 in vivo with LNCaP GRP-Pro cells. After almost two months of AZD0530 administration to castrated mice injected with LNCaP GRP-Pro cells, we observed a complete inhibition of metastasis by AZD0530. Although inhibition of primary tumor growth was not significant as reported by other researchers working on various cancers, AZD0530 demonstrated potent inhibition on tumor metastasis. None of the treated animals had metastases to regional lymph nodes but both surviving control animals did.

**Other Research Accomplishments**

We have characterized the expression of the NE induced expression of src, FAK and STAT3 in all major prostate cancer cell lines. We have also validated the action of
Src kinase inhibitor AZD0530 through the Src signaling pathway in two androgen-independent prostate cancer cell lines PC-3 and DU-145 by examining the status of phosphorylation of the downstream kinases and substrates. Through this study, we have identified the molecular mechanism of AZD0530. In vivo inhibitions of tumor progression by AZD0530 are also underway. These data are presently being combined for publication submission.

We have determined the downstream signaling cascades from NE activation and delineated the effect of a novel oral src kinase inhibitor AZD0530 at these signaling points. This is presently in preparation for publication.

**Key Research Accomplishments**

We have demonstrated that Src kinase is the key player in neuropeptide-mediated AR activation. Together with our studies in the chimeric growth of androgen-sensitive and androgen-insensitive cells, we are more confident with our proposed hypothesis. A paracrine effect exists for androgen insensitive CaP cells to support the survival and proliferation and migration of androgen sensitive CaP cells in a castrated environment. We have further delineated the impact of NE differentiation in prostate cancer.

**Reportable Outcomes**

**Abstract presentations 2006-2007**


3. 2006 Evans, C.P., Bai, L., Kung, H-J., and Yang, J.C. Androgen-sensitive prostate cancer survival and progression is supported by neuroendocrine prostate cancer cells. Urological Research Society, Salzburg Austria.

**Publications 2006-2007**


11. 2007 Evans CP. Editorial Comment on: Long-Term Intravesical Adjuvant Chemotherapy Further Reduces Recurrence Rate Compared with Short-Term Intravesical Chemotherapy and Short-Term Therapy with Bacillus Calmette-Guerin (BCG) in Patients with Non-Muscle-Invasive Bladder Cancer. Eur Urol. epub.


Conclusions
We have made headway into understanding the paracrine relationship between neuropeptide expressing, androgen-insensitive CaP cells and their ability to support the proliferation and migration of androgen sensitive CaP cells. Critically, we have identified src kinase as a molecule central to the process. We have been awarded a NIH CTEP phase II trial to study a novel, oral src kinase inhibitor AZD0530 in androgen-insensitive prostate cancer patients based upon our work.

References
None

Appendices
none