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Preface:

Annual progress report – Transcription Factor Stat3 in Metastatic Progression of Prostate Cancer.

Please, find enclosed my progress report for the first four months of grant # W81XWH-06-01-0076. This grant was originally funded to me in December 2005 while I was appointed as Assistant Professor at Georgetown University in Washington DC. In January 2006, I was recruited to Thomas Jefferson University and the transfer of this grant took more than one year. The grant transfer was completed (for this grant) on March 12th 2007, which is the official starting date for the work. Therefore, the grant report covers work performed from March 12th 2007 to June 30th 2007 (four months).

As we head into the fiscal year 2007-2008 of this project, I will take the opportunity to thank the Department of Defense Prostate Cancer Program for past and continued support.

Sincerely,

Marja Nevalainen, M.D., Ph.D.
Associate Professor
Cancer Biology
Kimmel Cancer Center
Thomas Jefferson University

Introduction:

The majority of prostate cancer fatalities are caused by development of androgen-independent growth of prostate cancer and metastatic spread of the primary tumor. There are currently no effective treatments for androgen-independent and metastatic prostate cancer. Moreover, the molecular mechanisms underlying progression of prostate cancer from primary tumor to metastasis remains a complex and poorly understood process. Identification of new therapeutic target proteins and identification of the molecular changes that lead to metastatic progression will be critical for development of better therapeutic intervention of prostate cancer and for development of an effective strategy to prevent progression of human prostate cancer.

The other major problem in clinical management of prostate cancer is a lack of reliable prognostic markers for identification of prostate cancers that are likely to progress to aggressive metastatic disease. Since the detection and diagnosis of early stage prostate cancers have

significantly improved over the last five years and since the clinical course of prostate cancer is highly variable, it is of utmost importance to develop effective prognostic markers to identify prostate cancers that are likely to progress aggressively to hormone-refractory and metastatic disease.

We propose to mechanistically test the central hypothesis that transcription factor Stat3 stimulates metastatic progression of human prostate cancer. Specifically, ***we hypothesize that*** both active Stat3 and IL-6 suppress homotypic adhesion and stimulate heterotypic adhesion, motility and invasion of prostate cancer cells *in vitro* and *in vivo*. Moreover, ***we propose that*** the stimulatory effect of IL-6 on metastatic progression is mediated through Stat3 signaling pathway. ***The third major hypothesis*** of the proposed work is that active Stat3 predicts clinical outcome of prostate cancer. In other words, we propose that active Stat3 will provide as a tumor biomarker for identification of prostate cancers that recur early and/or progress to metastatic lethal disease.

SPECIFIC AIMS:
(1) Establish whether active Stat3 inhibits homotypic adhesion and stimulates heterotypic adhesion of human prostate cancer cells *in vitro* and *in vivo*.
(2) Determine whether IL-6 stimulates heterotypic adhesion, invasion and motility of human prostate cancer cells, and establish the signaling pathways that mediate this effect.
(3) Determine whether activation of Stat3 in human prostate cancer predicts the clinical outcome of the disease.

We are pleased to report solid progress during the first 4 months of funding of this award. Specifically, we have started the Aim #3 of the grant which has generated valuable results towards the first publication. Moreover, we have made solid progress with the Aim I of the work proposed in this award. Specifically, we have established in the lab the wound filling assays and Boyden chamber assays for determination of cell motility. We expect to make significant progress for the Aims #1 and #3 of the grant during the fiscal year 2007-2008, which will result in a manuscript before the second year of the funding period ends.

Body:

Statement of Work:

Task 1. **Establish whether Stat3 suppresses homotypic adhesion of human prostate cancer cells *in vitro.* Months 1-18.**
a. Establish whether Stat3 suppresses homotypic adhesion of human prostate cancer cells *in vitro* using adenoviral gene delivery.
b. Establish whether Stat3 suppresses homotypic adhesion of human prostate cancer cells *in vitro* in stable prostate cancer cell lines expressing inducibly dominant-negative or wild-type Stat3.
c. Establish whether Stat3 suppresses homotypic adhesion of human prostate cancer cells *in vitro* using RNA interference.

Task. 2. **Determine the invasion-regulatory effect of Stat3 on established human prostate tumors in nude mice using adenoviral delivery. Months 13-24.**

a. Determine the invasion-regulatory effect of Stat3 on established human prostate tumors in nude mice using adenoviral delivery.

b. Establish the invasion-regulatory effect of Stat3 on human prostate tumors in nude mice using inducible expression of WT/DNStat3 in stable cell clones.

c. Determine the invasion-regulatory effect of Stat3 on established human prostate tumors in nude mice using adenoviral delivery.

Task 3. **Establish whether Il-6 stimulates heterotypic adhesion, motility and invasion of prostate cancer cells in vitro. Months 18-30.**

a. Subclone IL-6 cDNA into adenoviral expression vector.

b. Establish whether Il-6 stimulates heterotypic adhesion, motility and invasion of prostate cancer cells *in vitro.*

c. Identify the signaling pathway(s) that mediate the effects of IL-6 on adhesion and invasion of prostate cancer cells: Suppress IL-6 effect in prostate cancer cell by adenoviral gene delivery of DNStat3, DNMek1, DNAkt or DNStat5. Months 25-30.

Task 4. **Determine the *in vivo* effects of exogenous IL-6 on homotypic adhesion, invasion and metastasis of human prostate cancer cells. Months 25-36.**

a. Establish the effect of IL-6 that is administered to nude mice carrying orthotopically implanted prostate tumors.

b. Determine the effect of adenoviral expression of IL-6 on invasion and metastasis in orthotopically implanted prostate tumors.

Task 5. **Determine whether activation of Stat3 in human prostate cancer predicts the clinical outcome of the disease. Months 24-36.**

a. Immunohistochemical staining of the material I, II, II and IV.

b. Evaluation of the immunostainings.

c. Statistical analysis and photography.

Aim #3: Determine whether activation of Stat3 in human prostate cancer predicts the clinical outcome of the disease.

3a. Determine the independent prognostic value of activation of Stat3 in human prostate cancer.

3b. Determine whether activation of Stat3 in prostate cancer cells in patients with intermediate risk clinical features (Gleason grade 3 and 4) predicts early recurrence and poor prognosis.

Our ***working hypothesis 3a*** is that activation of Stat3 in human prostate cancer will predict unfavorable disease outcome. ***Working hypothesis 3b*** is that activation of Stat3 in prostate cancers of Gleason grade 3 and 4 predicts early recurrence and poor survival. We will test these hypotheses by applying our immunohistochemical staining method to detect active Stat3 in three independent materials of a total of 551 + 403 + 136 + 99 prostate cancer specimens with clinical follow-up data.

Statement of Work Related to Aim #3:

Task 5. **Determine whether activation of Stat3 in human prostate cancer predicts the clinical outcome of the disease. Months 24-36.**

a. Immunohistochemical staining of the material I, II, II and IV.
b. Evaluation of the immunostainings.
c. Statistical analysis and photography.

We have now immunostained the materials I, II, III and IV for active Stat3 and total Stat3. We are currently in the process of starting to score the tissue microarrays and run the statistical analysis to create the Kaplan Meyer curves for the patient survival analysis. Moreover, we have determined the frequency of constitutive activation of Stat3 in clinical human prostate cancer metastases to lymph nodes and to bones. Moreover, we have analyzed Stat3 activation in paired primary human prostate cancers and their distant metastases. Finally, we have determined the frequency of constitutive activation of Stat3 in recurrent human prostate cancer samples and in hormone-refractory recurrent human prostate cancer samples (please, see the Table 1 summarizing these results below).

Overall, a clear positive immunoreaction for active Stat3 was detected in 64% (84/131) of prostate cancer metastases (Table 1). In prostate cancer metastases to regional lymph nodes, an intense immunoreaction for active Stat3 was detected in 77% (51/66) of the specimens, while Stat3 was activated in 67% (10/15) of the bone metastases. Moreover, Stat3 was active in 56% (28/50) of prostate cancer metastases to other distant organs than bone. To further investigate Stat3 activation in advanced prostate cancer, we assessed Stat3 activation in recurrent human prostate tumors. Significant activation of Stat3 was detected in 86% (162) of 188 recurrent human prostate cancer specimens (Table 1). Of these 188 patients, 121 had been treated with androgen deprivation before the recurrence occurred. Stat3 was constitutively active in 96 of the 121 recurrent prostate cancers (79%) treated with hormone therapy (Table 1). In summary, our results indicate that Stat3 is constitutively active in the majority of distant prostate cancer metastases as well as in recurrent hormone-refractory clinical prostate cancer.

Table 1. *Stat3 activation in prostate cancer metastasis and in recurrent hormone-refractory prostate cancers.*

	No. of patients	**%**
Prostate cancer metastases (lymph node metastases, bone metastases and metastases to other organs):	131	100
Stat3 activation status;		
Negative	47	36
Positive	84	64
Prostate cancer metastases to regional lymph nodes:		
Stat3 activation status;	66	100
Negative	15	23
Positive	51	77
Prostate cancer metastases to bone:		
Stat3 activation status;	15	100
Negative	5	33
Positive	10	67
Prostate cancer metastases to other organs:		
Stat3 activation status;	50	100
Negative	22	44
Positive	28	56
Recurrent prostate cancers:		
Stat3 activation status;	188	100
Negative	26	14
Positive	162	86
Recurrent prostate cancers treated with hormone therapy:	121	100
Stat3 activation status;	25	21
Negative	96	79
Positive		

Key Research Accomplishments:

*** Determination of the frequency of constitutive activation of Stat3 in clinical samples of human prostate cancer metastases to lymph nodes and to bone.**

*** Determination of the frequency of constitutive activation of Stat3 in clinical samples of recurrent human prostate cancer samples and in hormone-refractory recurrent human prostate cancers.**

*** Establishment of in vitro assays for determination of prostate cancer cell motility.**

*** Detailed reportable accomplishments are listed below.**

Reportable Outcomes:

N/A at this time yet (four months into the funding).

C) Abstracts:

N/A at this time yet (four months into the funding).

Conclusions:

1) Transcription factor Stat3 is constitutively active in the majority of human prostate cancer metastases to lymph nodes and to bone.

2) Transcription factor Stat3 is constitutively active in the majority of recurrent human prostate cancers and in hormone-refractory human prostate cancers.