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Role of CDK5 as a Tumor Suppressor Gene in Non-Small Cell Lung Cancer

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14. ABSTRACT
Our goal in this project was to characterize *Cdk5* as a tumor suppressor gene in non-small cell lung cancer. We sought to examine this function in the context of two of the major oncogene-driven mechanisms of lung carcinogenesis, activation of *Kras* and *Egfr*. To accomplish this, we have generated a novel inducible autochthonous lung cancer mouse model, CE- $Cdk5^{f/f}$, in which mutant *Egfr* expression is doxycycline inducible, and *Cdk5* ablation is mediated by nasally instilled adenoviral-*Cre*. We also introduced $Cdk5^{f/f}$ into a KC lung cancer model, in which *Kras* is activated, and *Cdk5* is simultaneously ablated, by adenoviral-*Cre*. In both models, ablation of Cdk5 resulted in significant acceleration of lung tumorigenesis. Our confirmation in two in vivo models of clinically germane oncogene-driven lung cancer, involving the oncogenes $EGFR^{L858R}$ and $Kras^{G12D}$, that *Cdk5* deletion accelerates or promotes tumorigenesis, strongly indicate that *Cdk5* behaves as a tumor suppressor in lung adenocarcinoma tumorigenesis.

15. SUBJECT TERMS
Lung cancer, CDK5, tumor suppressor gene

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INTRODUCTION:

The proposed research program will elucidate the role of *Cdk5* in lung cancer and the suitability of this gene as a target for therapy by using novel modular inducible transgenic mice. Non-small cell lung cancer (NSCLC) is the most common cancer diagnosed in the United States. It has been estimated that greater than 220,000 new cases of lung cancer will be diagnosed in the United States in 2014 and lung cancer will be responsible for the most common cause of cancer deaths. Recent efforts to classify distinct molecular subtypes of lung cancer have led to the novel findings that greater than 50% of adenocarcinoma of the lung can be separated into distinct oncogene driver classes. We have shown that inhibition of CDK5 in preclinical models of leukemia, prostate, pancreatic and other cancers inhibits growth and/or metastasis. We have ablated *Cdk5* in a murine model of *Kras/p53* (KP) driven non-small cell lung cancer (NSCLC), and, remarkably, have made the serendipitous observation that lung tumors appeared much earlier and grew faster. Thus, unlike in other tumor types, mentioned above, *Cdk5* seems to have a tumor suppressor (TSG) role in NSCLC. This suggests that CDK5 controls signaling pathways that modulate the behavior of NSCLC, with prognostic and potential therapeutic relevance. We hypothesize that *CDK5* is a tumor suppressor gene in NSCLC, and that characterization of its tumor suppressor mechanism may identify new molecular targets in NSCLC. In particular, we hypothesize that CDK5 antagonizes KRAS signaling in NSCLC. We additionally speculate that loss of CDK5 function in NSCLC may result in resistance to EGFR inhibitors in *EGFR*-mutant NSCLC. Our studies in this one-year proposal will support more extensive, mechanistic studies in the future.

The original specific aims are below:

Specific Aim 1. *Cdk5-mediated signal transduction in Kras-driven NSCLC*.

Rationale: We hypothesize that *Cdk5* ablation augments NSCLC development by dysregulation of one or more signal transduction pathways. In NSCLC, numerous signal transduction pathways have been shown to be altered, very frequently including those pathways downstream of Ras. Many pathways can play a role in NSCLC development, and may be the effectors of *Cdk5* ablation in NSCLC development; a survey of all of these is far outside the scope of this proposal.

Study Design: We will pursue a limited candidate approach of whether ablation of *Cdk5* in lung epithelium activates Ras-dependent signal transduction pathways and a more global "gene signature" approach.

Specific Aim 2. *Effect of Cdk5 ablation on EGFR mutant-driven NSCLC*.

Rationale: Our initial observation that *Cdk5* ablation accelerates NSCLC development was made in a *Kras*-driven model. *KRAS* is mutated in only approximately 25-35% of human lung adenocarcinomas. Therefore, it is important to determine whether *Cdk5* ablation can also accelerate development in NSCLC development driven by other oncogenes. We have chosen to examine an *EGFR*-driven model of NSCLC, since 1) *EGFR* is mutated or amplified frequently in human lung adenocarcinoma, 2) EGFR employs the RAS signal transduction pathway, as well as other effector pathways, and 3) *EGFR* mutations correlate with sensitivity to EGFR inhibitors, raising the question of whether *Cdk5* ablation can modify this sensitivity.

Study Design: We will examine whether *Cdk5* ablation also accelerates tumorigenesis in a murine model of NSCLC driven by mutant $EGFR^{L858R}$.

KEYWORDS:

- Lung cancer
- CDK5
- Tumor suppressor gene

OVERALL PROJECT SUMMARY:

Progress is listed in relation to each specific task in the "Statement of Work" and highlighted by *italics* for the past year of this one year proposal.

Task#1 - Harvest of tumors in *Kras/p53* NSCLC model (months 1-6).

We have harvested these tumors and have them formalin-fixed and embedded in paraffin. Given limited tumor samples we still need to pursue generation of the appropriate tumors for bio-banking as snap frozen samples.

Task#2 - Isolation of pneumocytes, transformation in culture (months 1-8).
We were unable to complete the isolation of these cells for technical reasons.
Each of the steps for Tasks #3 and 4 below are partially/completely dependent on the steps above.

Task#3 - Assay of Kras pathway effectors (months 7-12)
As we cannot isolate the necessary primary pneumocyte cells from Task#2 and there is limited tumor material from the animals, we have decided not to proceed with this Task at this time.

Task#4 - Gene expression analysis (months 7-12)
4a. RNA isolation and gene expression arrays (months 7-8)
The tumor material from Task #1 was stored as FFPE and although possible to isolate RNA we have decided to proceed with RNA isolation and gene expression analysis of tumors from CCSP-rtTA/tetO- $EGFR^{L858f}$ (CE) mice below (Task #5). RNA isolation is proceeding but we have had issues with purity as determined by Bio-Analyzer from our SKCCC Microarray Core. We are currently attempting different purification methods.

4b. Gene expression array analysis, development of *Cdk5* knockout gene signature (months 9-12)
Pending RNA isolation above.

Task#5 - Development of $EGFR^{L858R}/Cdk5^{f/f}$ NSCLC model breeding, months (months 1-12)
5a. $EGFR^{L858R}/Cdk5^{f/f}$ NSCLC model breeding (months 1-12).
We applied for and obtained approval from the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center IACUC for the studies described in our DoD grant award (see Appendix for documentation approval). We then took the appropriate combination of mice and mated them to produce cohorts of CCSP-rtTA/tetO- $EGFR^{L858R}/Cdk5^{f/f}$ (CE- $Cdk5^{f/f}$) mice and control animals (CE- $Cdk5^{+/+}$ and CE- $Cdk5^{+/f}$). This genetic strategy requires two generations of crosses to generate the required genotypes. There have been no issues with producing the required numbers of mice.

5b. $EGFR^{L858R}/Cdk5^{f/f}$ NSCLC model tumor development and analysis (months 8-12)
CE- $Cdk5^{f/f}$ and control mice have been treated with intranasal Ad-Cre and then one week later placed on doxycycline drinking water (2 mg/ml) changed weekly. Mice where then monitored clinically on a 2-3x per week examination schedule. We did not observe any differences in clinical behavior or weights between CE- $Cdk5^{f/f}$ and control mice. Mice were also imaged non-invasively using micro-computed tomography (microCT) and tumor burden determined. CE mice do not produce discrete tumors, but are more characteristic of a diffuse opacification of the airspaces consistent with a lepidic growth pattern. This precluded us from documenting numbers and size of individual tumors. However, we were able to determine relative tumor burden in experimental cohorts as shown below in Table 1. Our preliminary data consists of two experiments but already demonstrates in a statistically significant fashion that genetic ablation of Cdk5 appears to increase tumor burden at 3 weeks post-induction of $EGFR^{L858R}$ ($p=0.017$ by Fisher's Exact test). We sacrificed these animals at 10 weeks per our original proposed plan and did observe any gross differences for the primary tumors. There were no metastases present in either group of animals. Tumor samples are currently being analyzed by a veterinarian pathologist currently for differences in histology.

Table 1. Tumor burden comparison between *CE- $Cdk5^{f/f}$* and control.

Data Analyzed	Control	*CE- $Cdk5^{f/f}$*	Total
Lesser Tumor Burden	9	1	10
Equal Greatest-Greatest Tumor Burden	1	4	5

Total	10	5	15

KEY RESEARCH ACCOMPLISHMENTS:

- *Generation of a novel inducible autochthonous lung cancer mouse model; CE- Cdk5$^{f/f}$.*
- *Confirmation in two models of clinically germane oncogene-driven lung cancer, EGFR^{L858R} and Kras^{G12D}, that Cdk5 deletion accelerates or promotes tumorigenesis. These in vivo data suggest that Cdk5 behaves as a tumor suppressor in lung adenocarcinoma tumorigenesis.*
- *Cdk5 deletion does not appear to promote EGFR^{L858R} lung tumors to metastasis. The caveat is we sacrificed the animals early well before any clinical signs of disease and thus cannot preclude the possibility that clinical metastases may have formed at a later time point.*

CONCLUSION:

During this one year of support we have not been able to been able to adhere to the timeline of our "Statement of Work"; namely because of technical issues with Task#1 and #2 (as detailed above in the **"Body"**). As a result of these technical issues, these have precluded us from pursuing Tasks #3 and #4 in full as we proposed originally. However, we have been very successful with Task #5 and have developed a novel inducible autochthonous lung cancer mouse model; *CE- Cdk5$^{f/f}$*. Characterization of this novel mouse model has shown in a statistically significant fashion that *Cdk5* deletion can accelerate *EGFR^{L858R}* lung tumorigenesis suggesting that *Cdk5* has tumor suppressor properties in lung cancer development. In addition, we have decided to make use of materials from Task #5 and perform gene expression analysis on the tumors we have present (as detailed above in the **"Body"**).

"So What"

Through the support of this DoD award, we have confirmed that loss of the gene *Cdk5* accelerates cancer growth in two mouse models of non-small cell lung cancer. This indicates that *Cdk5* has tumor suppressor properties in lung cancer. Our proposal has begun to examine the mechanism of this effect. Further work on this mechanism could identify molecular targets for lung cancer therapy. Potentially, CDK5 may also affect sensitivity to EGFR inhibitors such as erlotinib; if so, this study could allow more efficient selection of patients for such treatments.

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