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Chi3l1 Regulation of Checkpoint Regulating, Costimulatory, and Coinhibitory Molecules in Primary and Metastatic Lung Cancer

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14. ABSTRACT

Chitinase 3-like 1 (Chi3l1; also called **BRP-39** in the mouse and **YKL-40** in man), the prototypic CLP, is expressed in an exaggerated fashion in a variety of diseases characterized by inflammation, injury and remodeling. The levels of Chi3l1 are also increased the serum and or tissues from patients with a variety of cancers including lung cancer and their levels are inversely correlated with prognosis of the patients. However, the role(s) of Chi3l1 in the pathogenesis of lung cancer have not been defined. To begin to address this issue, we generated Chi3l1 null mutant (*Chi3l1*$^{-/-}$) and lung-specific YKL-40 overexpressing (OE) transgenic (YKL40 Tg) mice and evaluated them in models of metastatic and primary lung cancer. In the two years of grant period, we accomplished most of the major goals that we initially proposed. In this project, we successfully demonstrated that: 1) the regulatory role and mechanism of Chi3l1 in the expression of immune checkpoint molecules and its effect on tumor infiltrating immune cells in metastatic and primary lung cancer, 2) impressive therapeutic effect of anti-Chi3l1 neutralizing monoclonal antibody treatment on the development and or progression of melanoma lung metastasis and primary lung cancer, 3) the significant inhibitory effect of Poly(I:C), a strong RLH stimulant, in the development or progression of primary lung cancer, 4) a significant association between the serum or tissue levels of Chi3l1 and the clinical course of the primary lung cancer patients.

15. SUBJECT TERMS

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I. Introduction

Recent studies demonstrated that the levels of circulating Chi3l1/YKL-40 are increased in many malignancies including cancers of the prostate, colon, rectum, ovary, kidney, breast, glioblastomas and malignant melanoma (1-13). In these diseases, the levels of YKL-40 frequently correlate directly with disease progression and inversely with disease-free interval and survival (1-13). This is particularly striking in lung cancer where the serum and tissue levels of YKL-40 are impressively increased and correlate with adverse outcomes (14-17). However, the mechanisms of these inductive responses and the roles of Chi3l1/YKL-40 in disease initiation and progression have not been adequately defined. In addition, the utility of serum Chi3l1 assessments as biomarkers for these diseases and the value of interventions that alter Chi3l1 production and or effector responses as cancer therapeutics have not been appropriately addressed. To address these deficiencies we have evaluated the roles of Chi3l1 in primary and metastatic lung cancer. Our preliminary studies demonstrate that (1) Chi3l1/YKL-40 is expressed in an exaggerated manner in human lung cancer where it correlates inversely with survival but does not correlate with the mutations that drive these malignancies; (2) in murine models, Chi3l1 is sequentially induced in normal peritumor and tumor tissues during the early and later stages, respectively, of lung cancer development; (3) Chi3l1 induction via a semaphorin 7a-dependent mechanism plays a critical role in the generation of a metastasis permissive pulmonary microenvironment; (4) in metastatic models, Chi3l1 production and metastatic spread can be inhibited via RIG-like helicase (RLH) innate immunity (18). These findings led us to hypothesize that Chi3l1 plays a major role in the pathogenesis of primary and metastatic lung cancer by augmenting immune checkpoint inhibitors (ICPI; PD-1/PD-L1/L2 and CTLA4) and altering T cell co-stimulation. To address this hypothesis, following specific Aims has been established in this grant proposal:

Aim #1. Define the expression and roles of Chi3l1 in the development, growth and metastatic progression of primary lung cancer *in vivo***.**

Aim#2. Characterize the mechanism of Chi3l1 induction in primary and metastatic lung cancer and the regulation of these pathways by RLH innate immune activation and the RLH inhibitor NLRX1.

Aim #3. Characterize the roles of Chi3l1 in the induction/regulation of ICPI and their ligands and T cell co-stimulators in murine primary and metastatic lung cancer and the mechanisms of these regulatory events.

Aim #4. Determine if the levels of circulating or tissue Chi3l1 correlate with patient responses to immune checkpoint-based therapeutic interventions (ICPTI) and the relationships between the expression of Chi3l1 and molecules associated with Chi3l1-induced immunosuppression.

II. Keywords

Chi3l1, YKL-40, melanoma, lung metastasis, primary lung cancer, immune checkpoint molecules, PD-1/PD-L1/L2, CTLA4.

III. Major Accomplishments of Grant Support

Overall, we successfully accomplished the major aims that we originally proposed. Our studies generated a number of novel findings in multiple areas of lung cancer pathogenesis. Specifically, we identified that Chi3l1 is an important regulator of immune checkpoint molecule PD-L1/L2 in immune cells (macrophages) and in the lungs in animal model of primary and metastatic lung cancer. Neutralization of Chi3l1 with monoclonal neutralizing antibody that our laboratory developed effectively blocks expression of immune checkpoint molecule and the development and/or progression of lung cancer both in animal models of metastatic and primary lung cancers. In accord with animal findings, the expression of Chi3l1 was significantly increased in the patients with lung cancer and tumor cells are the major cells expressing Chi3l1. Higher levels of Chi3l1 expression was associated with worse progression (recurrence)-free survival (PFS or RFS) of lung cancer, specifically in adenocarcinoma, suggesting potential use of Chi3l1 as a predictive biomarker for this type of lung cancer. In the patients treated with immune checkpoint inhibitor (ICPI), elevated levels (more than 5% increase after ICPI treatment compared to the pretreatment levels) of serum Chi3l1 were also significantly associated with worse PFS. In conclusion, these studies demonstrated that Chi3l1 plays an important role in the pathogenesis of metastatic and primary lung cancers potentially through regulation of immune checkpoint molecule. These studies also highlighted that Chi3l1 can be used as a predictive biomarker for tumor progression and an effective therapeutic target for the patients with primary and metastatic lung cancer.

1. Define the expression, role and effect of Chi3l1 in tumor lung metastasis and primary lung cancer

(Specific Aim 1).

*A. Expression of Chi3l1 in animal models of metastatic and primary lung cancers (**Major Task #1, Aim1**).* The B16/F10 (B16) melanoma cells were prepared as we described previously (19), the cells were delivered to WT and YKL-40 Tg mice *via* tail vein injection. The level of Chi3l1 expression was positively correlated with the number of melanoma cells (Fig. 1A). The expression of Chi31l in the primary lung tumors (KP mice; $Kras^{G12D/+}/P53^{-/-}$) was also increased mainly in the macrophages and other inflammatory cells surrounding tumor at early stage (<12 weeks of tumor induct) of tumor development (Fig. 1B, Left panel). However, at advanced stage of tumor development (>20 weeks of tumor induction), tumor cells prominently express increased levels of Chi3l1 (Fig. 1B). These studies demonstrated that the levels of Chi3l1 expression is positively correlated with tumor burden and tumor cells acquired the ability to express Chi3l1 with progression of tumors.

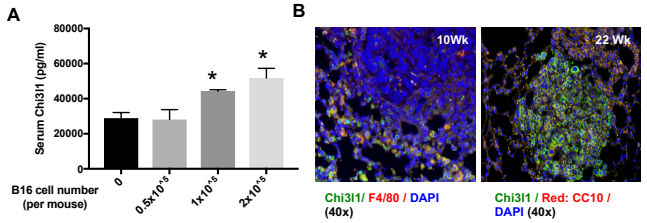


Fig.1. Chi3l1 expression is in animal model of lung cancers. (A) Serum levels of Chi3l1 were increased in a positive correlation with the number of melanoma cells challenged. (B) The kinetic changes Chi3l1 expression in primary lung cancer developed in KP mice. Left panel, IHC staining on the lungs of KP mice with 10 weeks after Cre induction. Right panel, IHC on 22 weeks of tumor induction. *p<0.05 compared to vehicle (PBS) control.

*B. Roles and effects of Chi3l1 in metastatic and primary lung cancers (**Major Task #1, Aim 1**).* Previously we have reported that melanoma lung metastasis was significantly diminished in the lungs of Chi3l1 null mice compared to wild type mice (19). On the other hand, transgenic overexpression of YKL-40 in the lung significantly enhanced melanoma colonization in the lung compared to WT animals (Fig. 2A). In the primary lung cancer model, the tumor development is also impressively enhanced in the mice with YKL-40 overexpression while tumor burden was decreased in the mice with Chi3l1 null mutation (Fig 2B). These studies demonstrated that Chi3l1 is required and also sufficient for the development and/or progression of both metastatic and primary lung cancer.

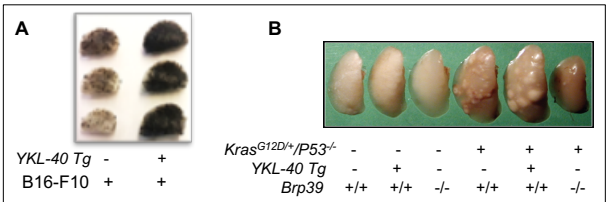


Fig.2. Chi3l1 is sufficient and required for the development and or progression of lung cancer. (A) Transgenic (Tg) overexpression of Chi3l1/YKL-40 increased the melanoma lung metastasis. (B) YKL-40 Tg overexpression enhanced lung cancer development in $Kras^{G12D/+}/P53^{-/-}$ (KP) mice while null mutation of BRP39 ($BRP39^{-/-}$) significantly decreased tumor burden. Panels A &B are the representative photographs out of at least 4 mice each group.

*C. Effect of Chi3l1 intervention using neutralizing anti-Chi3l1 antibody (**Major Task #2, Aim 1**).* For future therapeutic application, recently we used anti-Chi3l1 monoclonal antibody (called "FRG" antibody) that effectively block Chi3l1-stimulated MAPK/Erk and Akt activation and effector functions (data not shown). The effect of FRG treatment was assessed in animal models of melanoma lung metastasis and primary lung cancer models of KP or KPL ($Kras^{G12D/+}/P53^{-/-}$ /Luciferase) mice

Effect of FRG in melanoma lung metastasis. The anti-tumor effect of FRG antibody was first tested in *in vivo* animal model of melanoma lung metastasis as we reported previously (19). As shown in Fig 3. the antibody treatment impressively reduced melanoma lung metastasis in a dose-dependent manner compared to the mice treated with isotype control antibody (Fig. 3). FRG antibody treatment also significantly reduced the colony formation in the lung at post-treatment protocol that FRG was delivered after establishment of melanoma lung cancers, suggesting therapeutic effect of FRG on metastatic lung cancer development or progression (Data not shown).

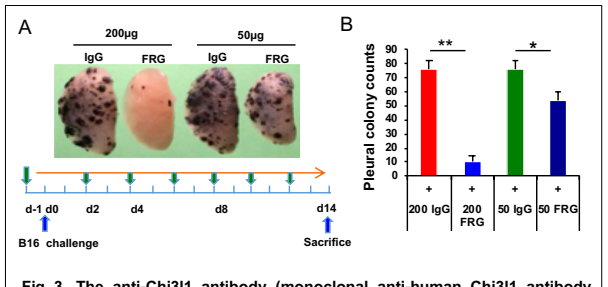


Fig 3. The anti-Chi3l1 antibody (monoclonal anti-human Chi3l1 antibody called FRG) effectively blocks melanoma lung metastasis. (A) WT mice were given high (200ug/mouse) and low (50ug/mouse) FRG or IgG control antibodies via i.p. injection, to melanoma challenged mice, every other day until sacrifice as indicated. (B) melanoma colony counts in the lung of mice treated with IgG control and FRG antibody.

Effect of FRG in the development or progression of primary lung cancer. To see the therapeutic effect of Chi3l1 intervention on primary lung cancer, WT and KP mice were treated with isotype control and FRG antibody for 13 weeks (i.p, 200µg/mouse, every other day) then the mice were sacrificed (Fig. 4A). As shown in Fig. 4B, lungs from the mice

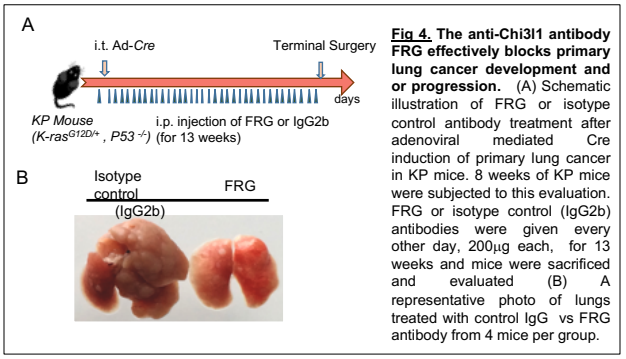


Fig 4. The anti-Chi3l1 antibody FRG effectively blocks primary lung cancer development and or progression. (A) Schematic illustration of FRG or isotype control antibody treatment after adenoviral mediated Cre induction of primary lung cancer in KP mice. 8 weeks of KP mice were subjected to this evaluation. FRG or isotype control (IgG2b) antibodies were given every other day, 200µg each, for 13 weeks and mice were sacrificed and evaluated (B) A representative photo of lungs treated with control IgG vs FRG antibody from 4 mice per group.

treated with FRG antibody showed significantly reduced primary tumor development compared to the mice treated with isotype controls. The effect of FRG on primary tumor development has been further tested using KPL mice ($Kras^{G12D/+}$/$P53^{-/-}$/luciferase). One week after adeno-cre instillation for induction of tumor development, KPL mice were treated with FRG antibody or isotype control IgG (5 mice/group; 200μg. every other day, i.p.) up to 10 weeks as illustrated in Fig. 5A. The tumor development and progression were monitored through in vivo imaging system that detect tumor luminescence. As shown in Fig. 5, KPL mice treated with FRG antibody showed no or significantly less tumor activity (luminescence) compared to the mice treated with isotype control antibody (Fig. 5, B and C). When viewed in combination, these studies suggest that neutralization of Chi3l1 using FRG antibody blocks development and or progression of melanoma lung metastasis as well as primary lung cancer. These studies highlighted the therapeutic potential of FRG antibody in lung cancer treatment.

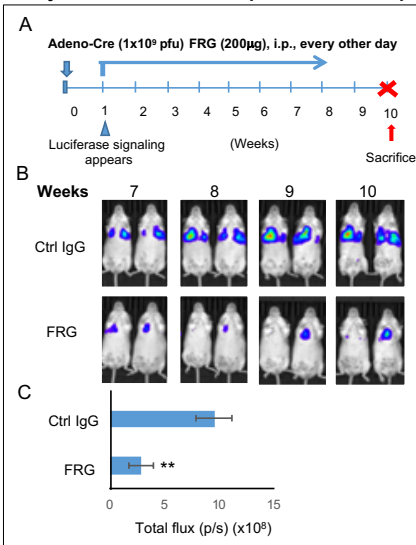


Fig 5. The anti-Chi3l1 antibody FRG effectively blocks primary lung cancer development and or progression. (A) Schematic illustration of FRG or isotype control antibody treatment after adenoviral mediated Cre induction of primary lung cancer in KPL mice. 8 weeks of KPL mice were subjected to this evaluation. FRG or isotype control (IgG2b) antibodies were given i.p., every other day, 200μg each, up to 10 weeks and mice were evaluated using in vivo imaging system (B) A representative photo of lungs treated with control IgG vs FRG antibodies from 5 mice per group. (C) Tumor burden in the region of interest (ROI) quantitated by total flux (p/s) of luminescence. **p<0.01.

Effect of FRG alone or in combination with anti-PD-1 antibody. Importantly, the degree of FRG inhibition of tumor development is comparable to anti-PD-1 treatment and they showed synergistic effect when both antibodies were used together (Fig. 6) These studies suggest that co-treatment of FRG antibody could enhance the therapeutic effects of anti-PD-1 treatment. These studies highlight potential therapeutic use of anti-Chi3l1 intervention in combination with immune checkpoint inhibitors in the treatment of lung cancer. These studies also suggest that developing bi-specific anti-Chi3l1/PD-1 or PD-L1 antibody could be a better option to maximize the therapeutic effect of lung cancer.

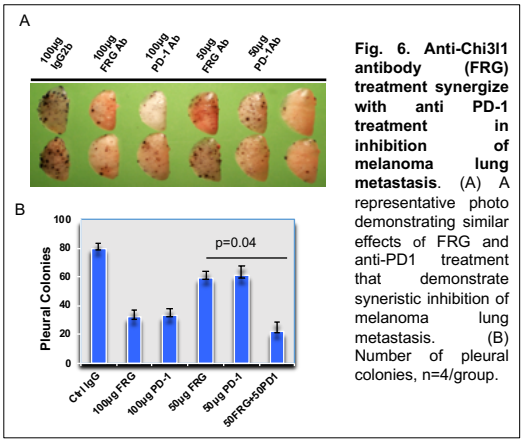


Fig. 6. Anti-Chi3l1 antibody (FRG) treatment synergize with anti PD-1 treatment in inhibition of melanoma lung metastasis. (A) A representative photo demonstrating similar effects of FRG and anti-PD1 treatment that demonstrate syneristic inhibition of melanoma lung metastasis. (B) Number of pleural colonies, n=4/group.

2. Characterize the role and effect of Chi3l1 in tumor infiltrating inflammatory cells with expression of immune check point molecules (ICPs) (Major Task 2, Specific Aim 1; Major Task 4 in Specific Aim 3). The Chi3l1 regulation of inflammatory cells and immune cells expressing immune checkpoint molecules (ICPs) such as PD-1, PD-L1 or CTLA4 are one of the major focus of this project. To begin to understand the role and effect of Chi3l1 in tumor infiltrating inflammatory and immune cells, we specifically evaluated the CD11b+ or CD68+ cells (targeting tumor associated macrophages) and CD8+ T cells (activated cytotoxic T cells) since these cells are reported as the most significantly implicated cells in the regulation of lung cancer development and progression in relation to the expression of ICPs (20, 21). Our studies demonstrate that Chi31l/YKL-40 regulates the expression of ICPs, since PD-L1, PD-L2 and CTLA4 expression levels are significantly increased in the lungs of YKL-40 Tg mice compared to WT controls (Fig. 7). In these mice, subset of Cd11b+/PD-L1 expressing cells were decreased in the cells isolated

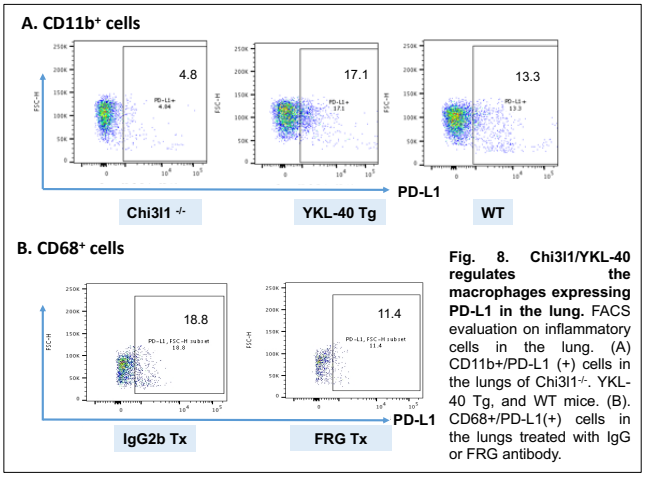


Fig. 8. Chi3l1/YKL-40 regulates the macrophages expressing PD-L1 in the lung. FACS evaluation on inflammatory cells in the lung. (A) CD11b+/PD-L1 (+) cells in the lungs of Chi3l1$^{-/-}$. YKL-40 Tg, and WT mice. (B). CD68+/PD-L1(+) cells in the lungs treated with IgG or FRG antibody.

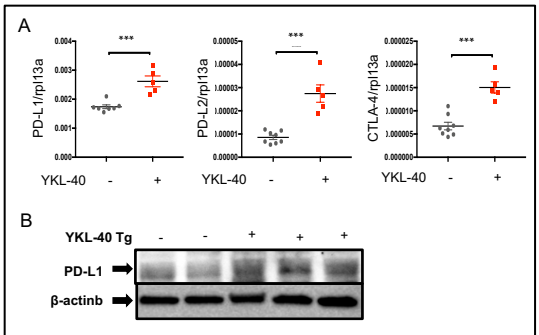


Fig. 7. Chi3l1/YKL-40 regulates the expression of immune checkpoint molecules in the lung. (A) RT-PCR on the immune checkpoint molecules in lungs from WT (YKL-40 Tg(-)) and YKL-40 transgenic (YKL-40 (+))mice. (B) Immunoblot on the lung lysates from WT and YKL-40 Tg mice. ***p<0.001.

from Chi3l1$^{-/-}$ mice while significantly increased in the cells from YKL-40 Tg mice compared to WT controls (Fig. 8A). further supporting a significant role and effect of Chi3l1/YKL-40 in the regulation of inflammatory cells expressing ICPs. It is also interesting to note that number of CD68+ PD-L1 expressing cells are also significantly reduced in the mice lung treated with FRG antibody compared to IgG control treated ones (Fig.8B). The immunohistochemical staining on the lungs with melanoma metastasis demonstrated that the lung of Chi3l1$^{-/-}$ mice showed significantly less number of Cd68+/PD-L1 positive inflammatory cell infiltration compared to WT animals, further supporting that Chi3l1 contribute to the recruitment of tumor-associated macrophages expressing PD-L1 (Fig 9A). Similarly, primary lung cancers developed in KP mice also showed increased levels of PD-L1 stained cells with YKL-40 overexpression (YKL-40Tg) compared to WT or null mutation of Chi3l1 (Chi3l1$^{-/-}$) (Fig. 9B). On the other hand, FRG treatment enhances CD8(+) T cell infiltration into primary tumor developed in KP mice compared to IgG control treated ones, suggesting a role of Chi3l1 in CD8 T cell infiltration into lung cancer (Fig. 10).

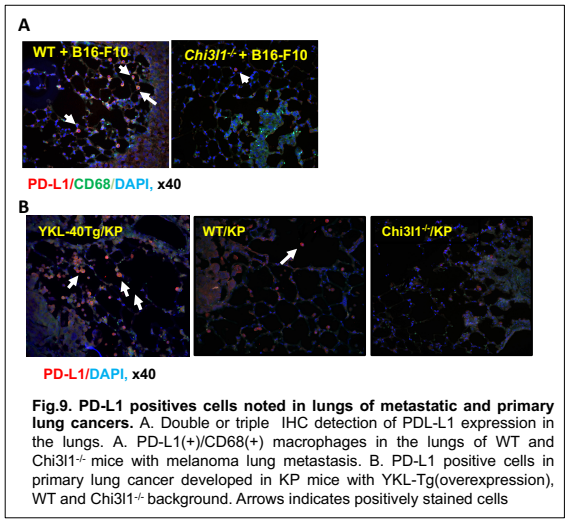
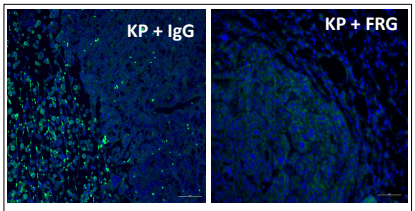


Fig.9. PD-L1 positives cells noted in lungs of metastatic and primary lung cancers. A. Double or triple IHC detection of PDL-L1 expression in the lungs. A. PD-L1(+)/CD68(+) macrophages in the lungs of WT and Chi3l1$^{-/-}$ mice with melanoma lung metastasis. B. PD-L1 positive cells in primary lung cancer developed in KP mice with YKL-Tg(overexpression), WT and Chi3l1$^{-/-}$ background. Arrows indicates positively stained cells



CD8/DAPI, x40 ; 10 weeks of Cre induction

Fig.10. A Representative microscope photo of CD8 immunostaining on the KP lungs treated with control IgG and FRG antibody.

To understand the mechanism of Chi3l1 regulation of PD-L1/L2 expression, we isolated macrophages from WT and Chi3l1 null mutant mice and stimulated IFN-γ since IFN-γ was well known stimulator of PD-L1/L2 expression (22). These studies demonstrated that IFN-γ-stimulated PD-L1/L2 expression was significantly reduced in the macrophages from Chi3l1 and IL-13Rα2 null mutant mice but not in the null mutant mice of TMEM219, Gal3 and mice treated with CRTH2 inhibitor Cay compound (Cay 10471, i.p. 30mg/kg, every other day) (Fig. 11 and data not shown). These studies demonstrated that Chi3l1 regulates IFN-γ-stimulated PD-L1/L2 expression via IL-13Rα2 but not through other putative receptors of Chi3l1 (23-26).

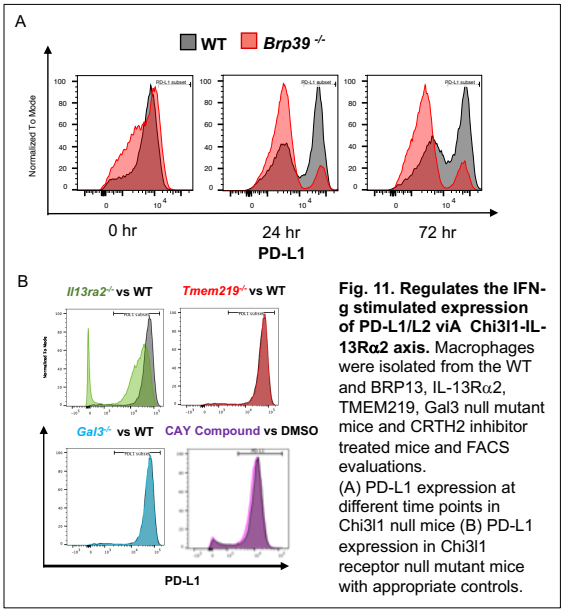


Fig. 11. Regulates the IFN-g stimulated expression of PD-L1/L2 viA Chi3l1-IL-13Rα2 axis. Macrophages were isolated from the WT and BRP13, IL-13Rα2, TMEM219, Gal3 null mutant mice and CRTH2 inhibitor treated mice and FACS evaluations.
(A) PD-L1 expression at different time points in Chi3l1 null mice (B) PD-L1 expression in Chi3l1 receptor null mutant mice with appropriate controls.

3. Characterize the mechanism of Chi3l1 induction in primary and metastatic lung cancer and the regulation of these pathways by RLH innate immune activation: Effect of Poly(I:C) treatment in the lung of KPL mice (Major Task 3, Specific Aim2). Previously, we reported that Poly(I:C) inhibits melanoma lung metastasis via regulation of expression of Chi3l1 by RLH innate immune activation (18). To see whether Poly(I:C) also inhibits the development and/or progression of primary lung cancer, KPL mice were treated with Poly(I:C) (5 mice /group, i.n, 30μg/mouse, every other day) and tumor immunoluminescence was monitored using in vivo imaging system. These studies showed significantly decreased tumor luminescence in the mice treated with Poly(I:C) compared to the mice with vehicle controls (Fig. 12), suggesting that RLH activation by Poly(I:C) could block Chi3l1 expression and contribution to primary lung cancer development similarly to melanoma lung metastasis as we previously reported (18) .

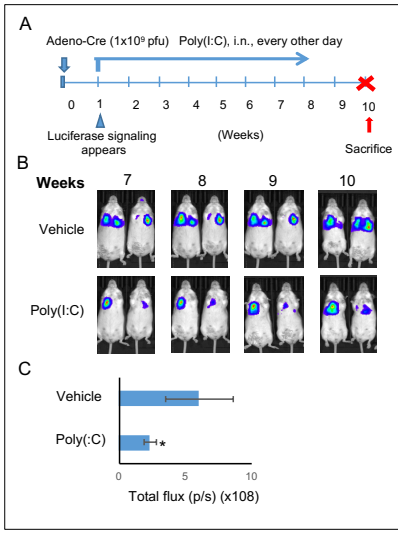


Fig. 12. The Poly(I:C) effectively blocks primary lung cancer development and or progression. (A) Schematic illustration of Poly(I:C) or vehicle control treatment after adenoviral mediated Cre induction of primary lung cancer in KPL mice. 8 weeks of KPL mice were subjected to this evaluation. Poly(I:C) or vehicle (PBS) were given i.n., every other day, 30 μg each mouse, up to 10 weeks and mice were evaluated using in vivo imaging system (B) A representative photo of lungs treated with control vehicle vs Poly(I:C) from 4 mice per group. (C) Tumor burden in the region of interest (ROI) quantitated by total flux (p/s) of luminescence. *p<0.05 compared to vehicle control.

4. Determine if the levels of circulating or tissue Chi3l1 correlate with patient responses to immune checkpoint-based therapeutic interventions (Major Task 5, Specific Aim 4). After we got HPRO approval, we initiated sample collection from the patients from Yale and Brown and started to evaluate serum levels Chi3l1 in the patients with and without immune checkpoint inhibitor treatment (anti-PD-1 or anti-PD-L1 immunotherapy). A total of 41 serum samples (38 paired samples, 3 pretreatment only samples) from Yale New Haven Hospital Yale University and 38 samples from Rhode Island Lifespan Hospital Brown University have been collected and subjected to the statistical analysis. Specifically, the correlations between the levels of Chi3l1 and patient's response to immune therapy (i.e. before and after anti-PD1 or anti-PD-L1 immunotherapy) including tumor progression, progression free survival (PFS) or overall survival (OS) were evaluated. The sample collection scheme is illustrated in Fig. 13A. The followings are the summary of the statistical evaluation on these samples:

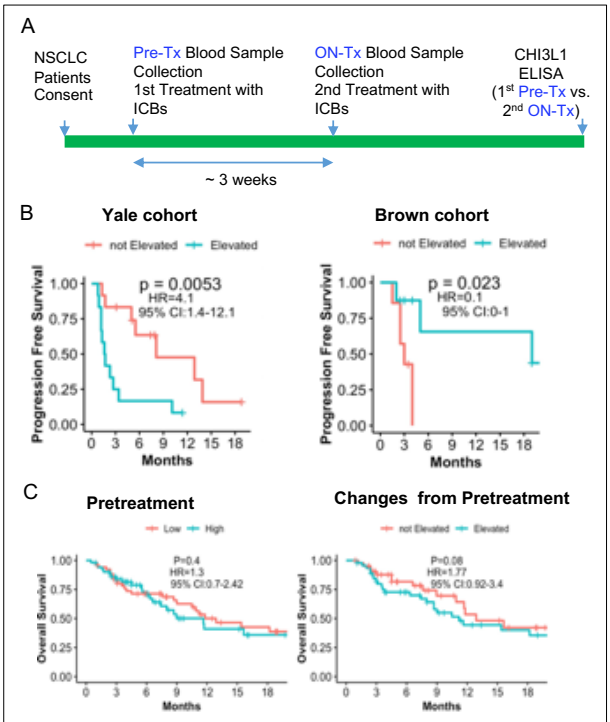


Fig.13. Evaluation on the associations between serum samples from the lung cancer patients treated with ICPI treatment. (A) Schematic illustration of sample collections from the patients. (B-C) Association between serum Chi3l1 levels and progression free survival (PFS) in Yale and Brown cohorts. (C) Association between serum Chi3l1 levels and overall survival (OS) in pooled samples.

a. The base line levels of Chi3l1 (pretreatment samples) are not significantly associated with the response (partial or complete remission) to ICPI treatment (data not shown)
b. Patients with more than 5% changes in the level of serum (regarded as "elevated" vs less than 5% as "not elevated") are associated with higher risk of progression (HR=4.1, 95% CI: 1.4-12.1) in Yale patients. On the other hand, elevated serum levels of Chi3l1 is associated with improved PFS (Fig. 13B) in Brown cohort, suggesting there is a significant heterogeneity of the samples between these two institutions.
c. For pooled samples, Chi3l1 levels at pretreatment were not associated with overall survival but elevated Chi3l1 seems to be associated with worse OS, with a trend towards significance (Fig. 13C).

When viewed in combination, these studies demonstrated that elevated serum levels of Chi3l1 showed decreased PFS, suggesting a possibility that the use of changes in serum levels of Chi3l1 as a predictive biomarker for the patients with ICPI treatment.

5. Automated Quantitative Analysis (AQUA) of Chi3l1 on patient's tissue samples (Major Task 5, Specific Aim 4). An anti-goat polyclonal anti-human Chi3l1 antibody (R&D Systems) was validated as detecting primary antibody suitable for AQUA staining. The antibody showed reasonable staining pattern for AQUA evaluation with 1:10 to 1:100 range of dilution, since Chi3l1 staining was well located either within epithelial cells (counterstained with Pan-cytokeratin stains) or other stromal cells (Fig. 14A). An archived lung cancer cohort of YTMA 250 were subjected to the AQUA staining with Chit3l1 and Pan-cytokeratin antibodies (*see* Appendices

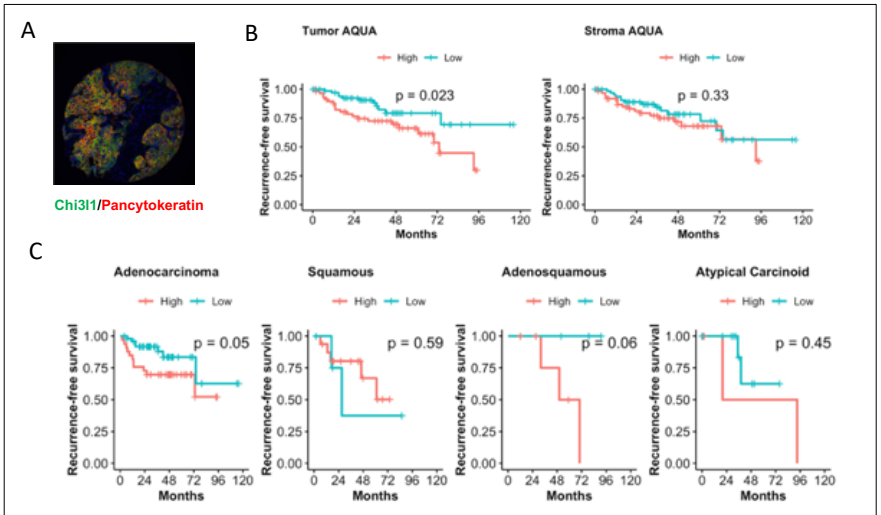


Fig.14. AQUA evaluation on the archived lung cancer samples. (A) A representative IHC staining with antibodies against Chi3l1 and pancytokeratin on tumor array. (B) Association between recurrence free survival (RFS) of the patients and Chi3l1 expression. (C) Association between RFS of the patients and Chi3l1 expression based on on histologic type of cancer.

for cohort characteristics). Overall, patients with high tumor expression of Chi3l1 (individuals with more than median AQUA score) showed less recurrence free survival (RFS) compared to the individuals with lower AQUA scores (individuals with less than median AQUA score) (Fig. 14B). On the other hand, stromal Chi3l1 expression did not show significant correlation with RFS (Fig. 14B). On the other hand, the overall survival was not significantly affected by the AQUA scores (data not shown). In subgroup analysis of patients based on histologic type of tumors, patients with adenocarcinoma is significantly associated (p=0.05) and adenosquamous carcinoma showed borderline significance of association (p=0.06), but squamous type is not associated with RFS (Fig. 14C). These studies suggest that tumor expression of Chi3l1 can be used as a predictive biomarker for the prognosis of the patients with mainly in adenocarcinoma patients. On the other hand, increased expression of Chi3l1 in stroma is trending toward association with better overall survival in adenocarcinoma patients (Data not shown).

IV. Impact

In the two years of grant period, we demonstrated that Chi31 is a critical mediator induced by tumor cells and significantly contributes to the development and progression in the lung cancer. Chi3l1 regulates tumor infiltrating inflammatory cells, especially macrophages and CD8 T cells expressing immune checkpoint molecules (ICPs), such as PD-1 and PD-L1, CTLA-4 that are frequently implicated in lung and other cancers. Newly developed anti-Chi3l1 antibody significantly decreases the development of metastatic and primary lung cancer to comparable levels of tumor inhibition achieved by PD-1 antibody treatment, and there is a synergistic inhibition when we put together. In accord to the findings of animal studies, studies in the lung cancer patients also demonstrated that the tumor expression of Chi3l1, that was inversely correlated with the progression or recurrence free survival (PFS or RFS) of lung cancer patients especially with adenocarcinoma. The elevated levels of serum Chi3l1 is also inversely correlated with progression free survival (PFS) in the lung cancer patients before and after ICPI treatment. These studies highlight that Chi3l1 is a potential predictive biomarker and also therapeutic target of lung cancer as a regulator of immune checkpoint molecules. Taken together, these studies provide strong supporting evidences for therapeutic use of neutralizing Chi3l1 antibody alone and or in combination with ICPI (anti-PD1 or anti-PD-L1) for the patients with primary or metastatic lung cancer.

V. Problems/Changes

1. Mouse breeding issue. Although we achieved most of the major goals proposed in the grant, we were not able to generate double mutant mice of KP or KPL with Sema7A or NLRX1 null mutation. We are speculating that the multiple mutant genes implicated in these crosses may cause a problem to generate the mice with desired genotypes. Since Poly(I:C) modulates the expression of Sema7a and NLRX1 expression (18), most works have been evaluated in the mice treated with Poly(I:C) as shown section #3 above.

2. Heterogenity issue in clinical samples due to compounding factors. Although only the lung cancer patients treated with ICPI's are included in current studies, we noticed that there are significant inter-sample (individual) variations potentially due to the many compounding factors that could affect the circulating or tissue levels of Chi3l1, such as cigarette smoking history, radiation or other drug treatment history, or the status of local or systemic infection. For accurate evaluation reflecting all these compounding factors, continuing sample collection will be necessary to have enough number patients to reach statistical significance.

VI. Products

1. Akosman B., Ma B., Kamle S., Lee C.M., Lee C.G. & Elias J. A. Chi3l1 Regulates immune checkpoint molecules in lung cancer. (27th Annual Pathobiology Graduate Program Retreat at Squantum Association, East Providence, RI, 2017)

2. B. Ma, S. Kamle, B. Akosman, C. He, C.G Lee, J.A. Elias. Inhibition of Melanoma Lung Metastasis by Chi3l1 Neutralizing Antibody. (Presented at Annual Meeting of the American Thoracic Society, San Diego, CA, May 2018).

3. Akosman B., Ma B., Kamle S., Kurtulus D., Lee C.M., Lee C.G. & Elias J. A. Chitinase-3-like-1: A novel

immunomodulatory target in lung cancer. (Presented at 29th Annual Pathobiology Graduate Program Retreat at Squantum Association, East Providence, RI, 2019; Abstract submitted to AACR Special Conference on Tumor Immunology and Immunotherapy conference, Boston, MA, 2019).

4. Akosman B., Kamle S., Ma B., Kurtulus D., Lee C.M., Lee C.G. & Elias J. A. Chitinase-3-like-1 regulates IFN-γ induced PD-L1 expression and anti-Chi3l1 treatment could be considered as a new immunotherapeutic agent in lung cancer. (Abstract submitted to 45th Annual New England Immunology conference, Woods Hole, MA, 2019).

5. Elias JA, LEE CG, and Kamle S. Bispecific antibodies against chi3l1 and pd1 with enhanced T cell mediated cytotoxic effects on tumor cells. Provisional US patent application:62/843931; 62/876507;898324

VII. Participants and other collaborating organizations:

Participants: *Information has not been changed from previous submission*

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VIII. Special Reporting Requirement: N/A

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