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TITLE: Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-Mesenchymal Transition: Implications for Pulmonary Fibrosis in Scleroderma

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14. ABSTRACT

Systemic sclerosis is a potentially devastating multisystem disorder characterized inflammation and autoimmunity, vasculopathy, and excessive extracellular matrix deposition. Treatment options are limited, nontargeted, potentially toxic and minimally effective, stressing the unmet need to advance of understanding of the pathogenesis with the hope of identifying novel therapeutic targets. Cadherin-11 is a type II mesenchymal cadherin. More recently, we have identified the expression of cadherin-11 on dermal and lung fibroblasts and its further upregulation by TGF-beta, a key cytokine in scleroderma pathogenesis. Herein we propose to investigate the role of cadherin-11 in modulating epithelial to mesenchymal transition of alveolar epithelial cells using both in vivo and in vitro approaches. Finally we will use a prospective cohort of scleroderma patients to determine if plasma levels of cadherin-11 levels are elevated in patients with interstitial lung disease and if these levels correlate with lung function.

15. SUBJECT TERMS

Nothing listed

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Grant DoD Award W81XWH-12-1-0516, Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-Mesenchymal Transition: Implications for Pulmonary Fibrosis in Scleroderma

INTRODUCTION.

My laboratory focuses on the potential role of cadherin-11 (Cad11) in fibrosis. We have previously reported that Cad11 expression is increased in fibrotic tissues from lungs of patients with idiopathic pulmonary fibrosis and skin of patients with systemic sclerosis. Subsequent studies have demonstrated that Cad11 is a critical mediator of lung and skin fibrosis using the intratracheal (IT) and subcutaneous bleomycin models. Preliminary studies suggest that Cad11 may regulate type 2 alveolar epithelial cell epithelial-to-mesenchymal transition (EMT), a process that contributes to the development of lung fibrosis. As opposed to the IT bleomycin lung fibrosis model, repeated administration of bleomycin via the intraperitoneal (IP) route is considered to better mimic human lung fibrosis and the process of EMT. This proposal builds on these recent observations and utilizes the IP bleomycin pulmonary fibrosis model. We hypothesize that Cad11 regulates the EMT in AEC during the development of pulmonary fibrosis and that cadherin-11 is therapeutic target in the intraperitoneal bleomycin model of pulmonary fibrosis. This proposal will be the first identify novel mechanisms by which Cad11 regulates EMT and build the foundation for additional translational studies seeking to develop Cad11 as a therapeutic target for SSc-ILD.

BODY

RESEARCH RESULTS

Regarding aim 1, we have acquired the Tomato Red SP-C-Cre, Rosa26 lacZ reporter mice and the breeding colony. These mice express Tomato Red under the direction of the SP-C promoter, and are supposed to be specific for type II alveolar epithelial cells. To induce lung fibrosis, these mice have been challenged with intraperitoneal bleomycin twice a week for four weeks. Lungs were harvested and frozen sections were obtained for dual color IF analyses (figure 1). AntiCadherin-11 antibody was used to stain the lungs (green) and type II alveolar epithelial cells appear red. The images in figure 1 demonstrate expression of cadherin-11 and SPC but the background levels are high. There are some cells that co-express these markers, suggesting that a subset of cadherin-11 expressing cells, likely fibroblast, are derived from SPC expressing type II alveolar epithelial cells. We are currently optimizing staining and fluorescent conditions as well as looking at expression of these markers during different time points in the model. The goal of these experiments is to track epithelial to mesenchymal transition during lung fibrosis, track the expression of cadherin-11 during fibrosis and determine if cadherin-11 plays a role in EMT during fibrosis.

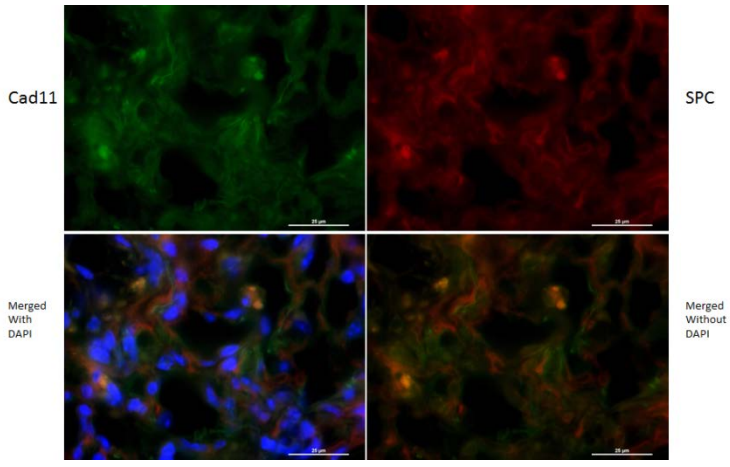

Figure 1. Tomato Red SP-C-Cre, Rosa26 lacZ reporter mice were given bleomycin to induce lung fibrosis. Lungs were also stained with anticadherin-11 antibodies (green) for IF analyses.

In the past year a lot of effort has been dedicated to the studies in Specific Aim 3. This aim seeks to determine the circulating levels of cadherin-11 in scleroderma patients with interstitial lung disease.

At the outset of these experiments, there was not a commercial Cad-11 ELISA. Therefore, we developed an ELISA and optimize our conditions using 2 anti-Cad-11 antibodies (clones 3H10 and 23C6). As seen in figure 2, our ELISA can detect both human and mouse soluble Cad-11.

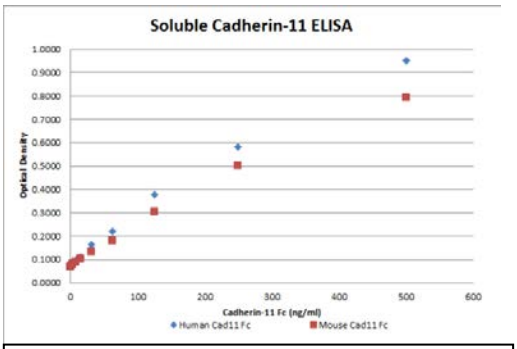

Figure 2. Soluble Cad-11 ELISA detects human (blue) and mouse (red) Cad-11

More recently, a commercial ELISA has become available (R&D Systems). We have obtained this ELISA and conducted experiments with it. This ELISA was first tested on a sera from healthy patients (n=20), systemic lupus erythematosus patients (n=29) and systemic sclerosis patients (n=20). Patients with lupus and systemic sclerosis both had an increase in circulating soluble cadherin-11 levels that was statistically significant over levels seen in healthy controls ($p<0.01$). As seen in figure 3, levels of cadherin-11 were more elevated in systemic sclerosis patients with the anticentromere antibody (ACA) and anti RNA polymerase III antibody (RNA POL). Furthermore, patients with other autoantibodies also had a remarkably elevated level. These data suggest that cadherin-11 levels are increased in patients with systemic sclerosis and lupus. These data use a small set of samples therefore, the goal in Aim 3 is to test a larger cohort of patients for serum cadherin-11 levels.

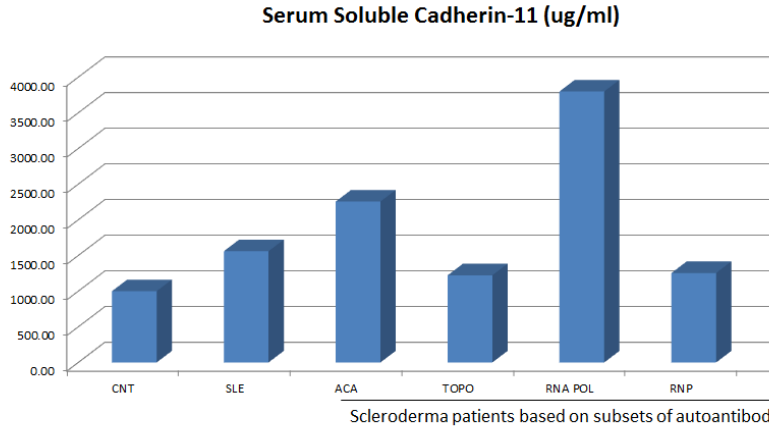

Figure 3. Serum cadherin-11 levels are increased in certain autoantibody subsets of systemic sclerosis using a commercial Soluble Cad-11 ELISA.

To further characterize the levels of soluble cadherin-11 in systemic sclerosis patients, we have obtained baseline serum samples from patients and healthy controls enrolled in the GENISOS study (UTHSC). Table 1 presents the basic demographics of the healthy controls and patients.

TABLE 1.

	Control	SSc
N	153	300
Age	48.8 +/- 14.2	50.1 +/- 13.1
Male (%)	26 (17%)	50 (17%)
	Age (49.2 +/- 14.4)	Age (49.7 +/- 15.2)
Female (%)	127 (83%)	250 (83%)
	Age (48.7 +/- 14.2)	Age (50.1 +/- 12.6)

Race (%)		
Caucasian (%)	71 (46%)	141 (47%)
Hispanic (%)	43 (28%)	85 (28%)
Black (%)	33 (22%)	64 (21%)
Asian (%)	0 (0%)	9 (3%)
Other (%)	6 (4%)	1 (<1%)

Table 2 presents the clinical characteristics of the systemic sclerosis patients at the time of enrollment in GENISOS.

TABLE 2

	Number of patients (total number 300)
Disease duration	3.95 +/- 2.93 years, range 0.17-17 yrs
Limited	125
Diffuse	171
Subset unknown	4
ANA positive	275
ANA negative	14
ANA not done	11
Anti-centromere positive	40
Anti-centromere negative	249
Anti-centromere not done/unknown	11
Anti-topoisomerase positive	46
Anti-topoisomerase negative	243
Anti-topoisomerase not done/unknown	11
Anti polymerase III positive	64
Anti polymerase III negative	225
Anti polymerase III not done/unknown	11

As seen in figure 4, patients with systemic sclerosis have a significantly increased level of soluble cadherin-11 relative to age/matched healthy controls.

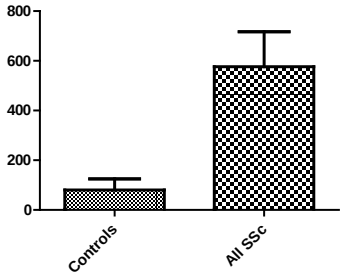

Figure 4. Serum cadherin-11 levels are increased in a large cohort of systemic sclerosis. P=0.0001

As seen in figure 5, patients with limited and diffuse forms of systemic sclerosis had a significantly increased level of soluble cadherin-11 relative to age/matched healthy controls.

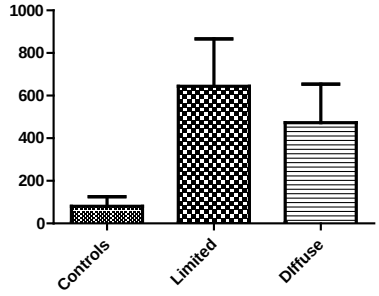

Figure 5. Serum cadherin-11 levels are increased in patients classified as either limited or diffuse scleroderma. P=0.0001
P<0.0001

We are currently performing additional analyses to determine if cadherin-11 levels are a biomarker for certain clinical subsets of systemic sclerosis and are predictive of the development of lung fibrosis in systemic sclerosis.

KEY RESEARCH ACCOMPLISHMENTS

1. Cadherin-11 deficient mice have decrease pulmonary fibrosis in the intraperitoneal model of pulmonary fibrosis
2. AntiCad11 antibodies are effective in treating lung fibrosis in the intraperitoneal model of pulmonary fibrosis this model
3. Cad11 regulates the in vitro TGF-beta induced epithelial-to-mesenchymal-transition (EMT) in MLE-12 cells, a mouse alveolar epithelial cell line.
4. Systemic sclerosis patients have increased levels of soluble circulating levels of Cad11.

REPORTABLE OUTCOMES.

None this year

CONCLUSIONS

Cadherin-11 is a mediator of lung fibrosis in scleroderma patients.

REFERENCES

None for current report

APPENDICES

None for current report

SUPPORTING DATA

No additional data for current report, see “BODY” section above for data.