Identification of Drivers of Tumor Lymphangiogenesis in Non-Small Cell Lung Carcinoma (NSCLC)

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ABSTRACT

Background: Non-small Cell Lung Carcinomas (NSCLCs) frequently spread to regional lymph nodes before they colonize other regions of the body, and the status of regional lymph nodes is an important prognostic factor for predicting the outcome of patients with lung cancer. It has recently been demonstrated that lymphangiogenesis, the sprouting of new lymphatic vessels from pre-existing vessels, facilitates the lymphogenous dissemination of NSCLC. However, the molecular mechanisms driving lymphangiogenesis in NSCLC are poorly understood.

Objective: Our aim was to identify novel lymphangiogenic genes by identifying lymphangiogenic lung tumor cell lines, and then to use microarray data to generate a "lymphangiogenic" gene signature.

Methods: Tumors from 13 lung tumor cell lines were stained with antibodies against LYVE-1 and Podoplanin. Lymphatic vessels were counted in 5 representative 20X fields per tumor. Average lymphatic vessel densities were then calculated. Cell lines were grouped into lymphangiogenic, non-lymphangiogenic, and intermediate categories. Microarray data from the two extreme groups were then compared to generate a "lymphangiogenic" signature.

Results: Four cell lines, (Calu-1, H1993, HCC461, and HCC827) displayed high intratumoral lymphatic density, and five cell lines (Calu-3, H1155, H1395, H1975, and H2073) displayed no intratumoral lymphatic vessels. The "lymphangiogenic" signature obtained from the microarray data from these groups contained 143 genes, including the lymphatic growth factor VEGF-C.

Conclusions: Our preliminary findings suggest that VEGF-C is an important driver of tumor lymphangiogenesis in NSCLC. The other 142 genes in the signature may also serve novel functions in regulating tumor lymphangiogenesis. Together, the results from this project provide mechanistic insight into the process of tumor lymphangiogenesis and metastasis. We believe that this information will lead to the development of new prognostic or predictive markers and therapeutic strategies to improve the outcome of patients with lung cancer.

INTRODUCTION

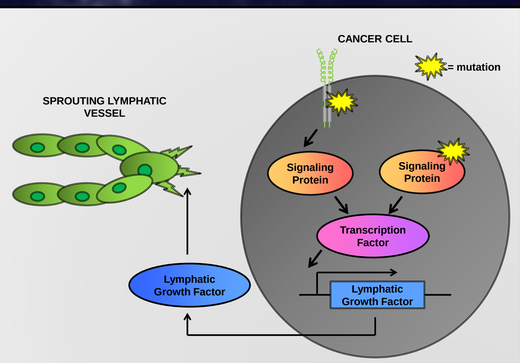

Figure 1. Model of tumor lymphangiogenesis. Tumors secrete factors which can induce lymphangiogenesis. This process facilitates metastasis to lymph nodes and potentially to distant organs. However, the array of lymphatic growth factors and the molecular mechanisms controlling their expression by cancer cells have not been fully delineated.

OBJECTIVES

I. Identify lung tumor cell lines that induce lymphangiogenesis
II. Use microarray data to generate a "lymphangiogenesis" gene signature.

METHODS

Immunofluorescence staining

Tumors from 13 lung tumor cell lines were stained with antibodies against Lyve-1 (R&D Systems, cat no. AF2125), podoplanin (abcam, cat no. ab11936) and smooth muscle actin (SMA) (Sigma, cat no. C6198). Slides were deparaffinized with xylene and rehydrated through a descending EtOH series. Antigen retrieval was performed with 0.01 M citric acid (pH = 6.0) in a pressure cooker. Slides were then washed with PBS and blocked for 1 hour with TBST + 20% Aquablock. Primary antibodies diluted in TBST + 5% BSA were then added and allowed to incubate overnight at 4˚ C. Slides were washed with TBST then secondary antibodies diluted in TBST + 5% BSA were added and allowed to incubate for 1 hour at room temperature. Slides were then washed again with TBST and coverslips were mounted with ProLong Gold plus DAPI.

Lymphatic vessel density measurements

Lymphatic vessels were counted in 5 representative 20X fields per tumor. Average lymphatic vessel densities were then calculated.

RESULTS

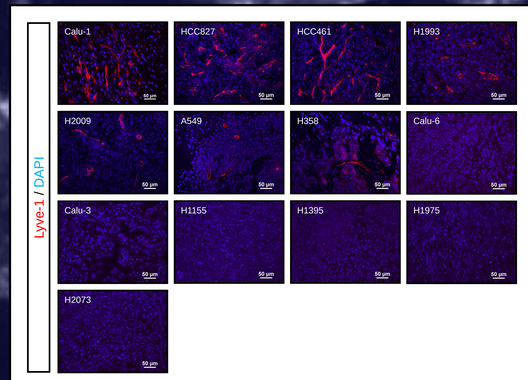

Figure 2. Immunofluorescence staining of lung tumors for Lyve-1. Lyve-1 is a receptor for hyaluronan and is highly expressed by lymphatic endothelial cells. Here we show representative images of tumor sections stained with an antibody against Lyve-1 (red) and counterstained with DAPI (blue).

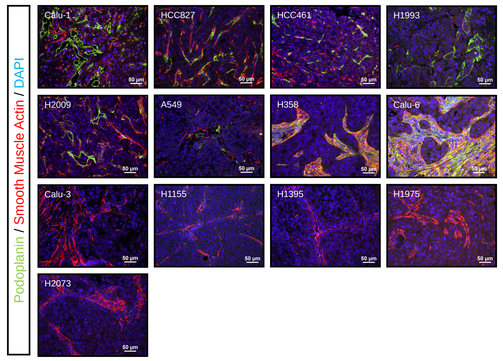

Figure 3. Immunofluorescence staining of lung tumors for podoplanin and smooth muscle actin (SMA). Podoplanin is a transmembrane glycoprotein highly expressed by lymphatic endothelial cells. SMA identifies fibroblasts. Here we show representative images of tumor sections stained with antibodies against podoplanin (green) and SMA (red) counterstained with DAPI (blue).

RESULTS

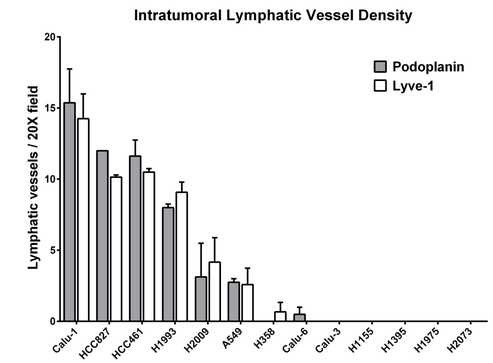

Figure 4. Graph showing the density of intratumoral lymphatic vessel density for the various lung tumors. The number of intratumoral lymphatic vessels were counted for Lyve-1 stained and podoplanin stained sections. The graph shows means ± SEM.

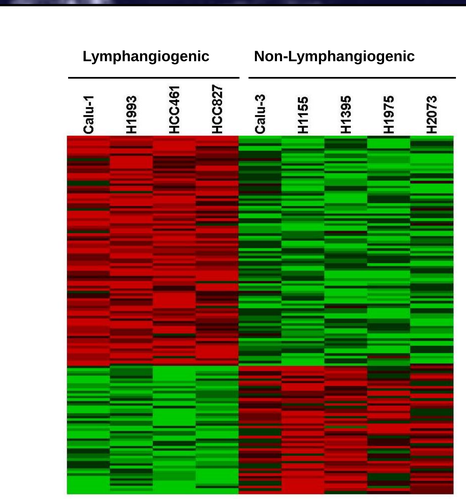

Figure 5. Tumor lymphangiogenesis gene signature. Microarray data for the various cell lines was analyzed to identify genes differentially expressed between "lymphangiogenic" and "non-lymphangiogenic" cell lines. 143 genes were found to be differentially expressed between "lymphangiogenic" and "non-lymphangiogenic" lung cancer cell lines. Each row represents a different gene. Upregulated genes appear red and downregulated genes appear green.

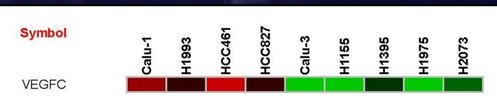

Figure 6. VEGF-C is differentially expressed between "lymphangiogenic" and "non-lymphangiogenic" cell lines. VEGF-C was part of the 143 gene signature shown in Figure 5. VEGF-C is approximately 30 fold higher in "lymphangiogenic" than "non-lymphangiogenic" cell lines.

RESULTS

		Lymphangiogenic				Non-Lymphangiogenic				
Symbol	Cytoband	Calu-1	HCC827	HCC461	H1993	Calu-3	Calu-6	H1155	H1395	H1975	H2073
VEGFC	4q34.3	2.4	2.1	3.1	1.8	1.3	1.9	2.6	1.7	1.5	1.7
VEGFC	4q34.3	2.4	2.2	3.1	1.9	1.3	1.9	2.4	1.7	1.5	1.7
VEGFC	4q34.3	2.1	2.2	3.0	1.8	1.3	1.9	2.4	1.7	1.5	1.7
VEGFC	4q34.3	2.3	2.2	3.1	1.8	1.3	1.9	2.6	1.7	1.5	1.8
VEGFC	4q34.3	2.1	2.1	3.1	1.8	1.2	1.9	2.4	1.7	1.5	1.7
VEGFC	4q34.3	2.1	2.1	3.1	1.8	1.2	1.9	2.4	1.7	1.5	1.7
VEGFC	4q34.3	2.3	2.2	3.1	1.8	1.2	1.9	2.6	1.7	1.5	1.7
VEGFC	4q34.3	2.3	2.2	3.1	1.8	1.0	1.9	2.4	1.6	1.5	1.7
VEGFC	4q34.3	2.1	2.1	3.1	1.8	1.0	1.9	2.4	1.6	1.5	1.6
VEGFC	4q34.3	2.1	2.1	3.1	1.8	0.9	1.9	2.4	1.6	1.5	1.6
VEGFC	4q34.3	2.1	1.9	3.1	1.8	1.0	1.9	2.4	1.6	1.5	1.5
VEGFC	4q34.3	2.1	1.9	3.1	1.8	1.0	1.9	2.4	1.6	1.5	1.5
VEGFC	4q34.3	2.3	1.9	3.1	1.9	1.3	1.9	2.4	1.7	1.5	1.6
VEGFC	4q34.3	2.3	1.9	3.0	1.8	1.0	1.9	2.4	1.6	1.5	1.5
VEGFC	4q34.3	2.1	1.9	3.0	1.8	1.0	1.9	2.3	1.6	1.6	1.6
VEGFC	4q34.3	2.3	1.9	3.0	1.8	1.2	1.9	2.3	1.6	1.6	1.6
VEGFC	4q34.3	2.1	1.9	2.8	1.8	1.1	1.9	2.3	1.6	1.6	1.5
VEGFC	4q34.3	2.1	1.9	3.0	1.8	1.0	1.9	2.3	1.6	1.6	1.5
VEGFC	4q34.3	2.3	1.9	2.8	1.8	1.1	1.8	2.3	1.6	1.6	1.6
VEGFC	4q34.3	2.3	1.8	2.8	1.8	1.2	1.8	2.3	1.6	1.6	1.6
VEGFC	4q34.3	2.1	1.8	2.8	1.8	1.1	1.9	2.3	1.5	1.6	1.5
VEGFC	4q34.3	2.1	1.8	2.8	1.8	1.1	1.9	2.3	1.5	1.6	1.5
VEGFC	4q34.3	2.1	1.8	2.8	1.6	1.2	1.9	2.3	1.6	1.6	1.5
VEGFC	4q34.3	2.3	1.8	2.8	1.6	1.2	1.9	2.2	1.6	1.6	1.5
VEGFC	4q34.3	2.3	1.8	2.9	1.8	1.3	2.0	2.2	1.6	1.6	1.7
VEGFC	4q34.3	2.3	1.8	2.9	1.8	1.3	2.0	2.2	1.6	1.6	1.8
VEGFC	4q34.3	2.3	1.8	2.9	1.8	1.3	2.0	2.2	1.6	1.6	1.7
VEGFC	4q34.3	2.3	1.8	2.9	1.6	1.3	2.0	2.2	1.6	1.6	1.5
VEGFC	4q34.3	2.3	1.8	2.9	1.6	1.3	2.1	2.2	1.6	1.6	1.5
VEGFC	4q34.3	2.3	1.8	3.1	1.8	1.3	2.1	2.2	1.6	1.6	1.7
VEGFC	4q34.3	2.3	1.8	3.1	1.8	1.3	2.1	2.2	1.6	1.6	1.7
VEGFC	4q34.3	2.3	1.8	2.9	1.6	1.2	2.0	2.2	1.6	1.6	1.5
VEGFC	4q34.3	2.1	1.8	2.8	1.6	1.2	2.0	2.2	1.6	1.6	1.5

Figure 7. VEGF-C copy number variations in lung cancer cell lines. Comparative genomic hybridization arrays revealed that VEGF-C can be amplified (red) in lymphangiogenic cell lines or deleted (green) in non-lymphangiogenic cell lines. We propose that these changes can influence VEGF-C expression by certain lung cancer cell lines.

CONCLUSIONS

I. Non-small cell carcinoma cell lines can be categorized as lymphangiogeneic or non-lymphangiogeneic based on Lyve-1 and Podoplanin/SMA staining.
II. 143 genes were found to make up the "lymphangiogenesis signature."
III. VEGF-C, an important driver of lymphangiogenesis, was found in the NSCLC "lymphangiogenesis signature".
IV. Copy number variation is one mechanism of altered expression of VEGF-C found in certain NSCLC cell lines.
V. We believe that this information will lead to the development of new prognostic or predictive markers and therapeutic strategies to improve the outcome of patients with lung cancer.

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