Human Pathology (2016) **54**, 174–183

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Elevated integrin α6β4 expression is associated with venous invasion and decreased overall survival in non–small cell lung cancer☆,☆☆

Rachel L. Stewart DO, PhD[a,b], Dava West MD[a], Chi Wang PhD[b,c], Heidi L. Weiss PhD[b,c], Tamas Gal PhD[d], Eric B. Durbin DrPH, MS[b,e], William O'Connor MD[a], Min Chen MD, PhD[b,f,1], Kathleen L. O'Connor PhD[b,g,*,1]

[a]*Department of Pathology, University of Kentucky, Lexington, KY 40536, USA*
[b]*Markey Cancer Center, University of Kentucky, Lexington, KY 40536, USA*
[c]*Department of Cancer Biostatistics, University of Kentucky, Lexington, KY 40536, USA*
[d]*Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23220, USA*
[e]*Department of Biomedical Informatics, University of Kentucky, Lexington, KY 40536, USA*
[f]*Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY 40536, USA*
[g]*Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY 40506, USA*

Received 21 December 2015; revised 26 March 2016; accepted 8 April 2016

Keywords:
Integrin signaling;
Cell adhesion;
NSCLC;
Pulmonary adenocarcinoma;
CD44

Summary Lung cancer carries a poor prognosis and is the most common cause of cancer-related death worldwide. The integrin α6β4, a laminin receptor, promotes carcinoma progression in part by cooperating with various growth factor receptors to facilitate invasion and metastasis. In carcinoma cells with mutant *TP53*, the integrin α6β4 promotes cell survival. *TP53* mutations and integrin α6β4 overexpression co-occur in many aggressive malignancies. Because of the high frequency of *TP53* mutations in lung squamous cell carcinoma (SCC), we sought to investigate the association of integrin β4 expression with clinicopathologic features and survival in non–small cell lung cancer (NSCLC). We constructed a lung cancer tissue microarray and stained sections for integrin β4 subunit expression using immunohistochemistry. We found that integrin β4 expression is elevated in SCC compared with adenocarcinoma ($P < .0001$), which was confirmed in external gene expression data sets ($P < .0001$). We also determined that integrin β4 overexpression associates with the presence of venous invasion ($P = .0048$) and with reduced overall patient survival (hazard ratio, 1.46; 95% confidence interval, 1.01-2.09; $P = .0422$). Elevated integrin β4 expression was also shown to associate with reduced overall survival in lung cancer gene expression data sets (hazard ratio, 1.49; 95% confidence interval, 1.31-1.69; $P < .0001$). Using cBioPortal, we generated a network map demonstrating the 50 most highly altered genes neighboring *ITGB4* in SCC, which included

☆ Competing interests: The authors have no conflict of interest to declare.

☆☆ Funding/Support: This work was supported by the National Institutes of Health grants T32 CA160003 (R. L. S.) and R01 CA109136 (K. L. O.), the National Center for Research Resources and the National Center for Advancing Translational Sciences grant UL1TR000117 (M. C.), the American Cancer Society Institutional Research grant IRG-85-001-25 (M. C.), and the Dr Joseph F. Pulliam Pilot Award (M. C. and R. L. S.).

* Corresponding author at: University of Kentucky, Markey Cancer Center, 741 S Limestone St, Lexington, KY 40506–0509, USA.
E-mail address: kloconnor@uky.edu (K. L. O'Connor).

[1] These 2 authors equally contributed as senior authors.

http://dx.doi.org/10.1016/j.humpath.2016.04.003

laminins, collagens, *CD151*, genes in the *EGFR* and *PI3K* pathways, and other known signaling partners. In conclusion, we demonstrate that integrin β4 is overexpressed in NSCLC where it is an adverse prognostic marker.

1. Introduction

Lung cancer is the leading cause of cancer-related death in the United States, with an estimated 158,040 deaths expected for the year 2015 [1]. Patients diagnosed with lung cancer have poor outcomes, with less than 17% of patients surviving 5 years [1]. Non–small cell lung cancer (NSCLC) is the most common form of lung cancer and can be further subdivided into a variety of histologic subtypes. These subtypes include adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large cell carcinoma. Lung SCC carries a poor prognosis and is typically treated with surgical resection, radiation, and traditional cytotoxic chemotherapy. Although a number of targeted therapies have recently been developed for the treatment of NSCLC, these agents target genomic alterations that occur more frequently in lung ADC, such as mutations in *EGFR* and rearrangements of *ALK* and *ROS1* [2,3]. SCC remains difficult to treat in part because of a lack of targeted therapies and because of its propensity for aggressive behavior.

The integrin α6β4 is an extracellular matrix receptor that has been implicated in carcinoma progression [4,5]. In normal epithelia, integrin α6β4 is expressed in the basal layer of cells where it binds to laminins in the extracellular matrix to nucleate the formation of stable adhesive structures termed *hemidesmosomes* [6]. In addition to serving an adhesive function, integrin α6β4 signaling is involved in many cellular processes including proliferation, survival, and wound healing [7–9]. The integrin β4 subunit (referred to herein as *integrin β4*) is particularly notable because of its long cytoplasmic signaling domain, which contributes to its ability to promote invasive and metastatic behavior in cancer cells [5]. During carcinoma progression, the integrin α6β4 is released from hemidesmosomes, which allows it to associate with the actin cytoskeleton [10]. Here, it activates RhoA, leading to membrane ruffling, lamellae formation, and the generation of traction forces [11]. These processes enable cell migration, thus allowing the cell to invade and metastasize [12]. In addition to its effects on cell motility, the integrin α6β4 cooperates with numerous growth factor receptors including EGFR, ErbB-2, ErbB-3, and c-Met to amplify downstream signaling to pathways such as PI3K, AKT, and MAPK (for review, see Stewart and O'Connor [5]). The integrin α6β4 is overexpressed in a wide variety of human cancers, where in many documented cases it positively associates with poor prognosis [5].

In carcinoma cells with mutant *TP53*, the integrin β4 promotes cell survival [7]. Interestingly, *TP53* mutations and integrin β4 overexpression co-occur in many aggressive malignancies including basal-like breast cancer, serous ovarian carcinoma, and pancreatic ductal ADC. Given that lung SCC has a high frequency of *TP53* mutations, we predicted that integrin β4 expression in this tumor type would associate with aggressive behavior and poor prognosis. Although integrin β4 expression in lung carcinomas has been studied previously, an association has not been demonstrated between integrin β4 overexpression and clinical outcomes [13–16]. We therefore investigated integrin β4 expression as it relates to histologic subtype, clinicopathologic features, and survival in NSCLC. Here, we report that integrin β4 expression is elevated in lung SCC and that its overexpression is associated with venous invasion and decreased overall survival in patients with NSCLC.

Table 1 Patient and tumor characteristics

n (% total)		
Patient characteristics		
Age (y), mean (range)		63 (39-84)
Sex		
Female	88	(42%)
Male	123	(58%)
Smoking status		
Total available	159	
Smoker	154	(97%)
Never smoker	5	(3%)
Residence		
Appalachian	145	(69%)
Non-Appalachian	59	(28%)
Out of state	7	(3%)
Vital status		
Alive	89	(42%)
Deceased	122	(58%)
Tumor characteristics		
Histology		
ADC	81	(38%)
SCC	99	(47%)
Other	31	(15%)
Differentiation		
Well	12	(6%)
Moderate	87	(41%)
Poor	112	(53%)
AJCC stage		
I	108	(51%)
II	37	(18%)
III	44	(21%)
IV	13	(6%)
Unknown	9	(4%)
Total	211	

Abbreviation: AJCC, American Joint Committee on Cancer.