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Wnt Pathway Activation Predicts Increased Risk of Tumor Recurrence in Patients with Stage I Non-Small Cell Lung Cancer

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Abstract

Objective—To determine the prevalence of Wnt pathway activation in patients with stage I NSCLC and its influence on lung cancer recurrence.

Background—Despite resection, the 5 year recurrence with localized stage I non-small cell lung cancer (NSCLC) is 18.4–24%. Aberrant Wnt signaling activation plays an important role in a wide variety of tumor types. However, there is not much known about the role Wnt pathway plays in patients with stage I lung cancer

Methods—Tumor and normal lung tissues from 55 patients following resection for stage I NSCLC were subjected to glutathione-S-transferase (GST) E-cadherin pull-down and immunoblot analysis to assess levels of uncomplexed β -catenin, a reliable measure of Wnt signaling activation. The β -catenin gene was also screened for oncogenic mutations in tumors with activated Wnt signaling. Cancer recurrence rates were correlated in a blinded manner in patients with Wnt pathway positive and negative tumors.

Results—Tumors in twenty patients (36.4%) scored as Wnt positive with only one exhibiting a β -catenin oncogenic mutation. Patients with Wnt positive tumors experienced a significantly higher rate of overall cancer recurrence than those with Wnt negative tumors (30.0% vs. 5.7%, $p=0.02$), with 25.0% exhibiting distal tumor recurrence compared to 2.9% in the Wnt negative group ($p=0.02$).

Conclusions—Wnt pathway activation was present in a substantial fraction of Stage I NSCLCs, which was rarely due to mutations. Moreover, Wnt pathway activation was associated with a significantly higher rate of tumor recurrence. These findings suggest that Wnt activation reflects a more aggressive tumor phenotype and identifies patients who may benefit from more aggressive therapy in addition to resection.

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INTRODUCTION

Lung carcinoma is the leading cause of cancer death in the United States as well as worldwide, and non small cell lung carcinoma (NSCLC) comprises 75–85% of lung cancers in the United States^{1, 2}. Unfortunately most patients are at an advanced stage at the time of diagnosis, and the overall 5-year survival rate for all lung cancer patients is 15%³. Patients with Stage I NSCLC, who undergo surgical resection, have the best 5 year survival rates. However, despite surgical resection, the 5 year recurrence in this group is 18.4–24%^{4–6}, and the 5 year survival rate is 58–73%⁷. Adjuvant chemotherapy has been investigated to improve the survival of this group, but the results have been controversial^{8–10}. The lack of better surgical cure for stage I patients and apparent absence of any significant benefit from adjuvant chemotherapy to improve survival has led to efforts to evaluate this group of patients for prognostic factors that may help to guide future therapy^{11, 12}.

Investigation of the roles of specific cellular pathways in NSCLC initiation and progression have revealed the frequent occurrence of mutations involving p53, K-ras, and EGFR^{13–15}. A number of studies have suggested that mutations in these genes are associated with advanced disease stage and poor prognosis. However, such findings have not been consistent^{11, 14–20, 20–24}. Wnt signaling plays critical roles in normal development as well as in post-natal tissue homeostasis through maintenance of stem/progenitor cells²⁵. Moreover, this pathway appears to regulate cell fate and differentiation in early lung development²⁶. A critical downstream effector in the canonical Wnt pathway is β -catenin, which is normally bound to E-cadherin at the inner surface of the cell membrane. A degradation complex comprised of glycogen synthase kinase-3 β (GSK-3 β), APC, and axin phosphorylates and processes unbound β -catenin for proteosomal degradation. Triggering of their cell surface receptors by secreted Wnt canonical family members leads to inhibition of the degradation complex resulting in β -catenin accumulation and translocation to the nucleus, where it participates as a transcription factor with TCF/LEF transcription factors in the activation of target genes^{25, 27}.

Aberrant Wnt signaling activation resulting from genetic alterations of intracellular components of this pathway plays an important role in the development of a variety of tumor types²⁸. Inactivating mutations of the APC gene are frequently observed in colorectal cancers, while mutations or in-frame deletions of exon 3 of the β -catenin gene, which prevent its phosphorylation and proteosome targeting have been observed in more than 90% of colon cancers and at lower frequencies in liver, endometrium, ovary, prostate, and stomach carcinomas^{29–34}. These genetic alterations result in constitutive activation of TCF transcription through the stabilization of β -catenin and the inappropriate activation of TCF target genes believed to be critical in tumorigenesis. More recently, Wnt canonical pathway activation has been observed in a substantial fraction of human breast, ovarian, and non-small lung carcinoma (NSCLC) cell lines. Levels of Wnt activation as measured by uncomplexed β -catenin or TCF transcriptional activation ranged from 5 to more than 100 fold higher than in normal or other tumor cells of the same tissue types^{35–40}. An autocrine mechanism involving over-expression of Wnt ligands and/or receptors, rather than APC or β -catenin mutations, was identified as the most frequent cause of pathway activation³⁹. Evidence that Wnt pathway downregulation caused specific inhibition of the proliferation of such tumor cells argues that Wnt signaling contributes importantly to the malignant phenotype^{38, 39, 41}. Thus, concerted efforts are underway to identify small molecule inhibitors and other approaches with which to therapeutically target this activated pathway in tumors⁴².

There is only limited evidence concerning the prevalence and clinical significance of Wnt pathway activation in tumors of NSCLC patients^{40, 43}. These studies have relied on the use

of immunostaining for β -catenin to assess Wnt pathway activity in NSCLC tissues and have led to conflicting findings concerning both prevalence and prognostic implications. We developed a biochemical approach to identify Wnt activation in primary NSCLC by comparison of uncomplexed β -catenin levels in tumor and normal lung tissues of the same patient and utilized this methodology to determine the frequency of Wnt pathway activation in stage I NSCLC and its influence on lung cancer recurrence.

METHODS

Patients

NSCLC tumor and normal tissues were obtained from the Tisch Cancer Institute Biorepository, Mount Sinai. We selected patients who underwent resection for stage I NSCLC from June 2006 to June 2008. All patients in this study were determined to be stage I based on pathological staging, in which they underwent mediastinal lymph node sampling including N1, upper and lower mediastinal nodes. Although pathological reports did not consistently specify the station numbers for N1 nodes being evaluated, each specimen had multiple N1 lymph nodes examined by the pathologist. Patients underwent preoperative staging with CT and PET scans and were deemed to be stage I by these criteria as well. Tissue specimens were linked to each patient by use of a unique number assigned to each patient. Paired samples of tumor and normal lung were obtained from each consented patient. None of the patients in the study had been given chemotherapy or radiation prior to surgery. We used prospectively collected data linked to this tissue bank to obtain information on patient demographics, co-morbidities, operative course, pathological staging and postoperative course. Inpatient and outpatient charts were reviewed, and patients were also contacted for additional follow-up information. Pathological staging was performed in accordance with the guidelines set forth by the American Joint Committee on Cancer (AJCC) ⁴⁴. The study was reviewed and approved by Mount Sinai's Institutional Review Board.

Tissue Samples

Tissues were immersed in Optimal Cutting Temperature (OCT) compound and frozen at -70°C . Hematoxylin and eosin (H&E) stained sections were reviewed by a pulmonary pathologist to confirm the diagnosis and quality of tissue, and 20 micron thick cryosections were obtained for analysis of tumor sections exhibiting the presence of more than 50% of tumor cells. Matched normal lung tissue scored as containing 100% normal cells from each patient served as a negative control.

Protein Extraction and Western Blot Analysis

Cryo-sectioned samples were homogenized in lysis buffer as previously described ⁴⁵ using mortar and pestle. Uncomplexed β -catenin, was measured by a capture assay using glutathione S-transferase (GST) fused to the β -catenin binding domain of E-cadherin synthesized in *E. coli* ⁴⁵. Immunoblot analysis of uncomplexed and total β -catenin was performed as previously described ⁴⁵ using mouse β -catenin primary antibody (BD Pharmingen, San Jose, CA, USA). Anti-mouse immunoglobulin G (IgG) secondary antibody conjugated to Alexa Fluor 680 was purchased from Amersham Bioscience (GE Healthcare, UK). Quantification of immunoreactivity was performed using the Licor Odyssey Imaging System (LI-COR, Lincoln, Nebraska, USA). For each set of paired normal and tumor samples, the capture assay and Western blot analysis were performed at least twice on consecutive sections of the same tissue with Wnt activated H23 NSCLC cells and Wnt negative 293T cells, respectively, serving as positive and negative controls.

Quantification of TCF mediated luciferase reporter activity

293T cells were infected with TOP-luciferase and renilla luciferase as previously described³⁹. Cells were plated in 10 cm plates at 1×10^6 cells/plate and treated 24 hours later with increasing concentrations of Wnt3a or control conditioned medium for 48 hours. Cells were then trypsinized, washed and resuspended in 10X the cell volume prior to lysis and processing for luciferase reporter assay using the dual luciferase reporter Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Luciferase reporter activity was calculated by dividing the TOP-luciferase by renilla luciferase. The rest of the cells were processed for Immunoblot analysis of uncomplexed and total β -catenin as previously described⁴⁵.

Sequencing of CTNNB1 exon 3

Genomic DNA was extracted from NSCLC tissue specimens using the DNeasy extraction kit (Qiagen, Maryland), and PCR amplified using primers flanking β -catenin (CTNNB1) exon 3 (forward 5'-TTGATGGAGTTGGACATG; reverse 5'-CAGCTACTTGGTTCTTGAG). This region of the β -catenin gene corresponds to the N-terminal phosphorylation domain that contains the sites of known β -catenin mutations in tumors⁴⁶. Gel purified PCR fragments were sequenced by the Mount Sinai DNA sequencing core facility.

Statistical Analysis

The clinical characteristics of patients with Wnt positive and negative NSCLCs were compared using the Student *t* test, the Wilcoxon test or chi-square test, as appropriate. Disease-free survival of patients with Wnt positive and negative tumors was determined by the Kaplan-Meier method. Disease-free survival was defined as the time from surgery to the first diagnosis of local, regional or distant disease recurrence, or until the last follow-up. We used Cox Proportional Hazards Regression Analysis to assess whether Wnt status was associated with disease free survival after controlling for other prognostic factors. The SAS 9.0 statistical package (SAS Institute, Cary, NC) was used for all statistical analyses. Two-tailed *p* value of less than 0.05 was considered significant.

RESULTS

To validate the capture assay for determination of uncomplexed β -catenin levels in cells or tissues as a surrogate for detection of Wnt activation, we exposed 293T cells to increasing Wnt3a concentration and compared the resulting increase in uncomplexed β -catenin as measured by the capture assay with levels of TCF transcriptional activation using a reporter for TCF signaling. Figure 1A shows that uncomplexed β -catenin levels increased as a function of increasing Wnt concentration while total β -catenin levels were little affected. Moreover, the increased levels of uncomplexed β -catenin correlated well but with a less dynamic range compared to the increasing levels observed in TCF reporter activity (Fig. 1B), a sensitive marker for Wnt pathway activity in living cells³⁹.

We next applied the capture assay to the analysis of activated Wnt signaling in paired stage I NSCLC and normal lung tissues. Figure 1C shows results with matched tumor and normal lung tissue samples from representative Stage I NSCLC patients. H23, a Wnt autocrine activated NSCLC line, and 293T, a Wnt negative immortalized human embryonic kidney line, served as positive and negative controls, respectively, in this analysis³⁹. It can be readily observed in independent tests that tumor tissue from patient #9 showed increased uncomplexed β -catenin levels when compared to normal lung tissue from the same patient. In contrast, patient #19 tumor and normal tissue showed no differences in uncomplexed β -catenin expression under the same conditions. For each tumor and normal tissue pair, we

also analyzed total β -catenin and actin levels as internal controls for the amount of protein utilized in the capture assay. Results of replicate analysis of the same samples were consistent (Fig. 1C, 2, and data not shown).

We tested Wnt activation by this approach in paired tumor and normal lung tissue samples from 55 stage 1 NSCLC patients. For each paired sample, two different observers scored results in the absence of any knowledge of clinical data. Using this approach, tumors from twenty patients (36.4%) exhibited elevated levels of uncomplexed β -catenin. Mutations at specific serine and threonine residues within the N-terminal domain of β -catenin inhibit its phosphorylation-dependent degradation⁴⁶. To investigate the presence of mutations in this region, we performed sequence analysis on tumors, which scored positive in the capture assay. Only one (5.0%) Wnt pathway activated tumor specimen contained a β -catenin mutation. This tumor exhibited a TCT to TGT mutation changing serine to cysteine in exon 3, a common site for β -catenin oncogenic activation^{46, 47}. Thus, the great majority of positive tumors were likely due to Wnt pathway upregulation by an autocrine mechanism previously identified in NSCLC lines³⁹.

To analyze the relationship of elevated Wnt activity to lung cancer recurrence, patients were divided into Wnt positive (+Wnt) and Wnt negative (–Wnt) groups prior to decoding of the clinical data relevant to each sample. Figure 2 shows that 6 out of the 8 tumors from patients with recurrence scored as Wnt activated. Quantitative densitometry confirmed that the ratio of uncomplexed β -catenin levels in tumor as compared to normal lung tissue from the same patient was greater than 2.5 for each tumor scored as +Wnt, while the ratio was 1.0 or less for the tumors scored as –Wnt (**Suppl. Fig. 1**). Patient and tumor characteristics for +Wnt and –Wnt groups are summarized in Tables 1 and 2. Overall, the mean age of the patients was 67.9 years, and the median smoking history was 40 pack-years. The majority had adenocarcinoma with a median tumor size of 2.0 cm. The two groups were statistically similar in their major characteristics, including age, gender, tumor size, and type of resection (Table 1). The mean follow up length was 24.6 and 21.6 months in + Wnt and –Wnt groups, respectively. The postoperative recurrence rate was 30.0% (n=6) in the +Wnt group and 5.7% (n=2) in the –Wnt group (Table 2; $p=0.02$). Disease-free survival was significantly better for –Wnt compared with +Wnt patients ($p=0.029$; Figure 3). Analysis of recurrences revealed that patients in the +Wnt group also had a significantly higher risk of distal recurrence compared to the –Wnt group (25.0% vs. 2.9%, $p=0.02$). The sites of tumor recurrence are shown in Table 3. Multiple regression analysis revealed that Wnt pathway activation was the only independent risk factor for recurrence ($p=0.04$) after adjusting for age, gender, type of resection, and stage.

A minority of NSCLCs were squamous cell carcinomas, and Wnt activation in these tumors occurred at lower frequency (2 out of 10 tumors) than was observed for adenocarcinomas (18 out of 43 tumors) as indicated in Table 2. We then performed statistical analysis of recurrence frequency in those patients with Wnt positive versus Wnt negative adenocarcinomas. The results also showed a higher rate of recurrence in patients with Wnt+ versus Wnt– adenocarcinomas (33.3% vs. 8.0%, $p=0.05$).

DISCUSSION

The present study analyzed the frequency of Wnt pathway activation in stage I NSCLC, the mechanisms involved, and the relationship of Wnt pathway activation to disease free survival. This is the first study to utilize uncomplexed β -catenin levels as a means to measure Wnt activation in this way. This biochemical assay is only semi-quantitative but improves on immunostaining, which cannot discriminate uncomplexed (active) from complexed β -catenin (inactive) and, thus, cannot quantify Wnt activation. When we

quantified immunoreactivity of uncomplexed beta-catenin in tissue lysates by use of the Licor Odyssey Imaging System, densitometric analysis revealed that the ratio uncomplexed β -catenin levels in tumor as compared to normal lung tissue of the same patient was greater than 2.5 for tumors scored as +Wnt, while the ratio was 1.0 or less for those tumors scored as -Wnt. Using this approach, Wnt pathway activation occurred frequently in patients with stage I NSCLC and was found to potentially be useful as a prognostic factor for cancer recurrence.

For surgically resected stage I NSCLC the 5 year recurrence is 18.4–24%^{4–6}. To date there has not been consistent evidence indicating a benefit of chemotherapy after surgery in patients with early stage NSCLC. Because of heterogeneity in recurrence rates among patients within the same stage, a reliable tumor molecular or genetic marker that predicted those patients likely to develop recurrent disease might identify a subset that would benefit from adjuvant chemotherapy. Several studies, based on microarray technology, have been performed to determine genetic profiles predictive of survival in NSCLC and to develop genomic approaches for stratifying risk^{48–50}. However, the identified survival-related genes have lacked consistency among these studies. This may be due to differences in microarray technique, samples analyzed, and/or statistical methods applied. Also, mRNA expression cannot always indicate which proteins are expressed or how their activities may be modulated.

Mutations of K-ras, EGFR and p53 are frequently observed in NSCLC and have been analyzed as predictive markers^{13–15}. Some studies have suggested that mutations which activate K-Ras or EGFR and inactivate p53 are associated with advanced disease stage and poor prognosis in NSCLC^{16–20}. However, such findings have not been consistent^{11, 14, 15, 21–24}. Moreover, most of these studies have involved analysis of heterogeneous groups of patients across different stages of disease.

Studies evaluating the role of these pathways as prognostic markers in early NSCLC have also resulted in conflicting findings^(11, 18, 21). Silini and colleagues reported that K-ras mutation has an adverse effect on survival in patients with stage I lung adenocarcinoma⁵¹. However, other studies showed no statistically significant difference in overall or cancer-specific survival in patients with stage I NSCLC^{52, 53}. Similarly, conflicting outcomes have been reported on the roles of EGFR and p53 as potential prognostic markers in early NSCLC^{11, 15, 24, 54}. Whereas K-ras mutations tend to occur most commonly in the adenocarcinomas of the lung in smokers, EGFR mutations appear to target adenocarcinomas arising in individuals who have never smoked and women^{55, 56}. These findings suggest that K-ras and EGFR mutations may each exclude substantial numbers of patients. The problems associated with use of p53 mutations as a prognostic marker are due to the heterogeneity in methodology for detecting the presence of p53 mutations, which have been reported in terms of both gene mutations and detection of protein expression. Also, a large spectrum of ‘hot spots’ makes mutational analysis of p53 gene difficult in the clinical testing.

There is a growing body of evidence for activation of Wnt pathway in NSCLC^{39, 40, 43}. Efforts to detect evidence of Wnt pathway activation in lung tumor tissues by immunostaining revealed different patterns of b-catenin expression including membranous, membranous-cytoplasmic, and cytoplasmic-nuclear, at frequencies, which were difficult to reconcile with the lack of b-catenin mutations detected in the same large series⁵⁷. The mechanism responsible for a much higher frequency of Wnt pathway activation in NSCLC than accounted for by pathway activating mutations in known intracellular components, β -catenin or APC^{58, 59} derives from demonstration of autocrine mechanism in as many as 40–50% of NSCLC tumor lines³⁹. Of note, treatment of such tumor cells with Wnt antagonists, DKK1 or FRP, which act at the cell surface to inhibit Wnt/receptor interaction, resulted in

downregulation of activated Wnt signaling associated with inhibition of NSCLC proliferation and acquisition of a more differentiated phenotype³⁹. In our present study, primary NSCLCs showed a high prevalence of Wnt pathway activation as measured by elevated levels of uncomplexed β -catenin with 36.4% exhibiting increases compared to matched normal lung tissues. Yet, only 1 patient out of 20 with activated Wnt signaling exhibited a β -catenin oncogenic mutation, consistent with previous findings that such mutations are rare events in NSCLC^{39, 59, 57}. The high prevalence of Wnt pathway activation in NSCLC tissues analyzed by us strongly supports the conclusion that a Wnt autocrine mechanism is responsible in the large majority of Wnt activated primary NSCLCs.

Using immunostaining approaches, previous studies indicated that increased expression of β -catenin predicted a favorable prognosis in patients with NSCLC after resection^{60–62}. Hommura and colleagues reported a trend toward improved survival in patients with tumors having higher β -catenin expression. Multivariate analysis also showed that high β -catenin expression was a significant and independent favorable prognostic factor ($p=0.007$)⁶⁰. Another report indicated that reduced β -catenin expression in surgically treated NSCLC was associated with lymph node metastasis and an unfavorable prognosis⁶¹. In contrast, other studies have suggested that overexpression of the Wnt pathway was associated with poor tumor differentiation and tumor progression^{40, 43}. Immunostaining for Wnt 1 ligand expression led to the conclusion that Wnt1 positive status was a significant prognostic factor for recurrence in NSCLC patients ($p=0.013$). Wnt1 expression was also found to correlate with the expression of reported Wnt pathway targets, including c-Myc, vascular endothelial growth factor-A (VEGF-A), and matrix metalloproteinase-7 (MMP-7)⁴³. All of these studies evaluated the level of the Wnt pathway activity using immunohistochemical staining for beta-catenin protein, which is not quantitative and may be confounded by the presence of inactive, complexed β -catenin. In the present study, those patients identified as having increased Wnt pathway activity by the capture assay exhibited a higher rate of recurrence than those whose tumors showed no Wnt activation. Moreover, the pattern of recurrence in the +Wnt group favored distal metastasis with 5 patients (25.0%) in this group developing metastases distally compared to 1 patient (2.9%) in the –Wnt group. These results are consistent with a study by Nguyen and colleagues, who analyzed the role of Wnt pathway in determining distal NSCLC metastasis to the brain and bone. They found that Wnt activation, through HOXB9 and LEF1 target genes, was associated with metastasis to multiple organs in mouse models and humans. Also, reduction of Wnt activity attenuated the ability to form brain or bone metastasis in a mouse model⁶³.

There are some limitations in our study. Although our analysis showed that Wnt pathway activation significantly increases the risk of recurrence after resection of stage I NSCLC, sample size used in the study was relatively small. Thus, larger studies will be necessary to confirm our observations. Also, follow up of the patients potentially was not long enough to detect all recurrences despite the fact that the mean follow up time of around 2 years should capture most recurrences. In fact, new localized malignant lesions more than 2 years after resection of early lung cancers are more likely due to new primary malignancies than recurrences^{4, 64}.

Adjuvant chemotherapy has been shown to benefit patients with stage II-III NSCLC. However, the benefit for stage IB patients is controversial^{8–10}. The Stage IA patient group does not appear to benefit from adjuvant therapy after surgical resection⁶⁵. Our study is the first utilizing a quantitative means of identifying Wnt activated primary tumors to measure the level of Wnt pathway activity and correlate it with clinical outcomes. An increased level of Wnt pathway activation was associated with a statistically significant increased risk of tumor recurrence after surgical resection. Thus, Wnt pathway activation may have an important role in guiding adjuvant therapy in stage I patients. Investigating potential benefits

of adjuvant chemotherapy in such patients with stage I NSCLC could be an important step in efforts to improve outcomes of stage I NSCLC patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Uncomplexed b-catenin level is a valid marker for Wnt activation in non-small cell lung cancer (NSCLCs). 293T cells transduced with TOP-luciferase and renilla luciferase lentiviruses were treated for 48 hours with increasing concentration of Wnt3a conditioned medium. (A) Glutathione S-transferase (GST)-E cadherin capture assay as described in the Methods was performed on the treated cells. Total cell lysates (20 μg) and the GST-E-cadherin precipitates from 800 μg of cell lysate were subjected to immunoblot analysis with a mAb directed against β-catenin. (B) TCF reporter luciferase activity was performed on 1:10 of cell volume from the same Wnt3a conditioned medium treated cells as described in Methods. Luciferase reporter activity was calculated by dividing the TOP-luciferase by renilla luciferase (RL). (C) β-catenin capture assay performed with two representative human paired NSCLC and normal lung tissues in two independent experiments. Total cell lysates (800 μg) were subjected to GST-E cadherin capture assay as described in Figure 1A. T denotes tumor specimen and N denotes normal lung tissue from the same patient. H23, a Wnt autocrine activated NSCLC line, and 293T, an immortalized human embryonic kidney line, were used as positive and negative controls, respectively.

Figure 2.

Wnt pathway activation as measured by the β -catenin capture assay in NSCLCs from patients that developed recurrence. T denotes tumor specimen and N denotes normal lung tissue from the same patient. H23, a Wnt autocrine activated NSCLC line, and 293T, an immortalized human embryonic kidney line, were used as positive and negative controls, respectively.

Figure 3.

Disease-free survival according to Wnt pathway activity as measured by the b-catenin capture assay in tumors of 55 patients with pathologic stage I NSCLC. Included were 20 tumors scored as +Wnt and 35 tumors that scored as -Wnt in this assay.

Table 1

Demographic and clinical patient characteristics

Variable	+ Wnt (n = 20)	– Wnt (n = 35)	p Value
Age, years (median, IQ range)	68.0 (15.5)	70.0 (12.0)	0.70
Sex (male/female)	8:12	17:18	0.54
Race (No.)			0.71
White	18 (90.0%)	33 (94.3%)	
Black	1 (5.0%)	0	
Other	1 (5.0%)	2 (5.7%)	
Smoking, pack-years (median, IQ range)	40.0 (32.5)	40.0 (53.0)	0.97

IQ = interquartile range;

Table 2

Tumor characteristics and surgical intervention

Variable	+ Wnt (n = 20)	– Wnt (n = 35)	p Value
Tumor size, cm. (median, IQ range)	2.0 (1.9)	2.1 (1.6)	0.69
Histologic type (No.)			0.31
Adenocarcinoma	18 (90.0%)	25 (71.4%)	
Squamous cell	2 (10.0%)	8 (22.9%)	
Other	0	2 (5.7%)	
Differentiation *			0.79
Well	6 (31.6%)	6 (19.4%)	
Moderate	7 (36.8%)	17 (54.8%)	
Poor	6 (31.6%)	8 (25.8%)	
Stage (No.)			0.96
IA	13 (65.0%)	23 (65.7%)	
IB	7 (35.0%)	12 (34.3%)	
Resection Type			1.00
Lobectomy	15 (75.0%)	26 (74.3%)	
Segmentectomy	1 (5.0%)	3 (8.6%)	
Wedge	4 (20.0%)	6 (17.1%)	
# Stations examined (mean ± SD)	4.3 ± 1.5	4.1 ± 1.6	0.68
# Nodes examined (median, IQ range)	10.0 (8.5)	8.0 (7.0)	0.56

* n = 19 and n = 31 respectively; IQ = interquartile range;

Table 3

Long-term outcome

Variable	+ Wnt (n = 20)	– Wnt (n = 35)	p Value
Mean follow-up (m., mean $\pm$ SD)	24.6 $\pm$ 8.3	21.6 $\pm$ 7.4	0.17
Recurrence (No.)	6 (30.0%)	2 (5.7%)	0.02
Distant recur. (No.)	5 (25.0%)	1 (2.9%)	0.02

SD = standard deviation;

Table 4

Postoperative recurrence pattern

Recurrence Pattern	+ Wnt (n=6)	- Wnt (n=2)
Locoregional		
Lung	1	1
Distant		
Brain	2	0
Adrenal glands	0	1
Lung	1	0
Bone	2	0