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Wnt1 overexpression promotes tumour progression in non-small cell lung cancer

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ABSTRACT

Background: The *Wnt* gene family is involved in embryogenesis and tumourigenesis. We investigated the clinical significance of Wnt1 expression in non-small cell lung cancer (NSCLC).

Method: We studied 216 NSCLC patients. Immunohistochemistry was performed to investigate the Wnt1 expression in relation to the expression of β-catenin and Wnt-targets, including c-Myc, Cyclin D1, VEGF-A and MMP-7. The Ki-67 proliferation index and the intratumoural microvessel density (IMD) were also evaluated.

Results: The ratio of tumours with an aberrant β-catenin expression was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours ($p < 0.0001$). The Wnt1 expression significantly correlated with the expression of c-Myc ($p < 0.0001$), Cyclin D1 ($p < 0.0001$), VEGF-A ($p = 0.0160$), MMP-7 ($p < 0.0001$), the Ki-67 index ($p = 0.0048$) and the IMD ($p = 0.0267$). Furthermore, the Wnt1 status was a significant prognostic factor for NSCLC patients ($p = 0.0127$).

Conclusions: The Wnt1 overexpression is associated with the expression of tumour-associated Wnt-targets, tumour proliferation, angiogenesis and a poor prognosis in NSCLCs.

1. Introduction

Non-small cell lung cancer (NSCLC) is one of the most common human cancers with a poor prognosis. However, recent molecular biology studies have revealed that many molecules affect the various biological behaviours of malignant tumours. It is therefore considered important to clarify these tumour biology mechanisms in order to improve the clinical outcome of NSCLC patients.[1] The *Wnt* gene family encodes the multi-functional signalling glycoproteins that are involved in the regulation of a wide variety of normal and pathological processes, including embryogenesis, differentiation and tumourigenesis.[2,3] The *Wnt* genes have been classified into functional groups with separate downstream signalling pathways.[4] Amongst them, *Wnt1* stimulates the canonical Wnt/β-catenin signalling pathway, which leads to changes in cell fate and/or cell transformation.[5] The canonical Wnt/β-catenin signalling pathway regulates the transcription of the Wnt-target genes with TCF/LEF1 motifs.[6] The Wnt-target genes include various molecules associated with tumourigenesis.[7–10]

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As a result, the intratumoural Wnt1 expression could affect various biological functions through these Wnt-target genes. In fact, previous clinical studies have demonstrated that the Wnt1 expression is frequently upregulated in many human cancers.[11,12]

Therefore, the Wnt1 overexpression could affect tumour biology during tumour progression and understanding the biological mechanisms of the Wnt1 expression could lead to the development of a new treatment for cancer patients. Therefore, in order to clarify the biological significance of the Wnt1 expression in NSCLCs, we investigated the intratumoural Wnt1 expression in relation to its target molecules, including c-Myc,[7] Cyclin D1,[8] vascular endothelial growth factor-A (VEGF-A)[9] and matrix metalloproteinase-7 (MMP-7).[10] In addition, we also evaluated the tumour proliferation rate using the Ki-67 index, and tumour angiogenesis using CD34 staining.[1]

2. Materials and methods

2.1. Clinical characteristics of the patients

From January 1996 to December 2002, consecutive NSCLC patients who underwent surgery at the Department of General Thoracic Surgery, Breast and Endocrinological Surgery of Kagawa University were studied. This study was approved by the institutional review board of Kagawa University (14-7, a clinical study of biological markers in non-small cell lung cancers), and a signed informed consent was obtained from each patient. Tumour-node-metastasis (TNM) staging designations were made according to the postsurgical pathological international staging system. The lymph node status was pathologically evaluated using specimens resected by either thoracotomy or mediastinoscopy. In total, 216 patients with lung cancer up to stage IIIB, which included 123 patients with adenocarcinomas, 83 patients with squamous cell carcinomas and 10 patients with large cell carcinomas, were investigated (Table 1). The patients' clinical records and histopathological diagnoses were fully documented. This report includes follow-up data as of October 2007. Systemic chemotherapy using mitomycin/vinblastin/cisplatin or carboplatin/paclitaxel was performed in all patients with stages II–III NSCLCs: neoadjuvant chemotherapy in 43 patients and postoperative adjuvant chemotherapy in 50 patients. Radiation therapy was performed in 26 patients: twelve patients with T3 or T4 status and 14 patients with mediastinal lymph node metastases.

Table 1 – Demographic and clinical characteristics of patients

Characteristics	Number of patients	Percent
Total number of patients	216	100
Age, years		
Median	68	
Range	38–82	
Gender		
Male	154	71.3
Female	62	28.7
Smoking status		
Non-smoker	75	34.7
Smoker	141	65.3
Smoking pack-years		
Median	36	
Range	0–150	
ECOG performance status		
0	139	64.3
1	52	24.1
2	25	11.6
Histology		
Adenocarcinoma	123	56.9
Squamous cell carcinoma	83	38.4
Large cell carcinoma	10	4.7
Pathological stages		
I	123	56.9
II	27	12.5
III	66	30.6
Method of surgical resection		
Pneumonectomy	20	9.3
Lobectomy	179	82.8
Segmentectomy	9	4.2
Wedge resection	8	3.7
Chemotherapy	93	43.1
Neoadjuvant therapy	43	19.9
Postoperative adjuvant therapy	50	23.2
Radiotherapy	26	12.0

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

2.2. Immunohistochemical assays

We performed immunohistochemical studies to evaluate the intratumoural expression of Wnt1, β-catenin, c-Myc, Cyclin D1, VEGF-A and MMP-7, tumour proliferation rate using the Ki-67 index and tumour angiogenesis using CD34 staining. The following antibodies were used, along with isotype antibodies as negative controls: a rabbit polyclonal antibody for Wnt1 (H-89, Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted at 1:200, a mouse monoclonal antibody for β-catenin (C19220, Transduction Laboratories, Lexington, KY) diluted at 1:200, a mouse monoclonal antibody for c-Myc (9E10, Santa Cruz) diluted at 1:100, a mouse monoclonal antibody for Cyclin D1 (DSC-6, DAKO, Glostrup, Denmark) diluted at 1:200, a rabbit polyclonal antibody for VEGF-A (A-20, Santa Cruz) diluted at 1:200, a rabbit polyclonal antibody for MMP-7 (AB19135, Chemicon, Temecula, CA, USA) diluted at 1:300, a mouse monoclonal antibody for the Ki-67 antigen (MIB-1, DAKO) diluted at 1:40 and a mouse monoclonal antibody for CD34 (NU-4A1, Nichirei Corporation, Tokyo, Japan) diluted at 1:10.

Formalin-fixed paraffin-embedded tissue was cut into 4-μm sections and mounted on poly-L-lysine-coated slides. Sections were deparaffinised and rehydrated. The slides were then heated in a microwave for 10 min in a 10-μmol/l citrate buffer solution at pH 6.0, and cooled to room temperature. After quenching the endogenous peroxidase activity with 0.3% H_2O_2 (in absolute methanol) for 30 min, the sections were treated for 2 h at room temperature with 5% bovine serum albumin to block non-specific staining. Duplicate sections were incubated overnight with the primary specific antibodies.

Slides were then incubated for 1 h with biotinylated anti-rabbit IgG (Vector Laboratories Inc., Burlingame, CA) for Wnt1, VEGF-A and MMP-7 and biotinylated anti-mouse IgG (Vector Laboratories Inc.) for β-catenin, c-Myc, Cyclin D1, Ki-67 and CD34. The sections were incubated with the avidin-biotin-peroxidase complex (Vector Laboratories Inc.) for 1 h, and antibody binding was visualised with 3,3′-diaminobenzidine tetrahydrochloride. Lastly, the sections were lightly counterstained with Mayer's haematoxylin. Sections of lung tumours known to express Wnt1, c-Myc, Cyclin D1, VEGF-A or MMP-7 were used as positive controls for the immunohistochemical staining, respectively.

All the immunostained sections were reviewed by two authors (Huang and Ueno) who had no knowledge of the patients' clinical status. Cases with discrepancies were jointly re-evaluated, and a consensus was reached. For the expression of Wnt1, c-Myc, Cyclin D1, VEGF-A and MMP-7, five areas were selected at random and scored in cases with multiple areas of low intensity. Also, one random field was selected in sections where all staining appeared intense. At least 200 cells were scored per ×40 field about tumour cells. Regarding the β-catenin expression, we used the classification of staining patterns as reported previously:[13] (1) a membranous pattern, if immunoreactivity was present solely at the cell membranes; (2) a membranous-cytoplasmic pattern, if immunoreactivity was also present in the cytoplasm; (3) a cytoplasmic pattern, if immunoreactivity was chiefly present in the cytoplasm and in less than 20% of the nuclei and (4) a cytoplasmic-nuclear pattern, if immunoreactivity was present in the cytoplasm and concomitantly in more than 20% of the nuclei. The percentage of carcinoma cells with a positive staining for Ki-67 in a given specimen was scored as the Ki-67 proliferation index. For microvessel quantification, the three most highly vascularised areas detected by CD34 immunostaining were initially selected under the ×40 field, and a ×200 field (0.785 mm^2 per field) was used to count vessels in each of these areas. The average of three ×200 field counts was recorded as the intratumoural microvessel density (IMD).[1]

2.3. Statistical analysis

Because the distributions of seven values, including the percentages of Wnt1-positive tumour cells ($p = 0.2128$), c-Myc-positive tumour cells ($p = 0.1390$), Cyclin D1-positive tumour cells ($p = 0.1390$), VEGF-A-positive tumour cells ($p = 0.0531$), MMP-7-positive tumour cells ($p = 0.0875$), the Ki-67 proliferation index ($p = 0.0875$) and the IMD ($p = 0.1727$), showed normal distributions (Kolmogorov–Smirnov analysis), the statistical significances regarding these values were assessed by the *t*-test, ANOVA with Bonferroni/Dunn test or Pearson's correlation coefficient. The sample was classified as a Wnt1-positive tumour when the percentage of Wnt1-positive tumour cells was >50% because of the most significance in relation to the Ki-67 proliferation index, which is the same as reported previously.[14]

Overall survival was defined as the time from the treatment initiation (surgical resection, chemotherapy or radiation) to the date of death from any cause. The Kaplan–Meier method was used to estimate the probability of overall survival as a function of time, and differences in the survival of subgroups of patients were compared by using Mantel's log-rank test. A multivariate analysis was performed using the Cox regression model to study the effects of different variables on survival. All *p* values were based on the two-sided statistical analysis, and a *p* value of <0.05 was considered to indicate statistical significance.

3. Results

3.1. Wnt1 expression in NSCLCs

The Wnt1 expression appeared in the form of a cytoplasmic staining pattern (Fig. 1A). The Wnt1 expression was low in the normal alveolar epithelium. In contrast, regarding the intratumoural Wnt1 expression, the percentage of Wnt1-positive tumour cells varied greatly amongst the 216 NSCLCs (median, 45.5%; mean ± SD, 46.7 ± 27.9%). One hundred and six carcinomas (49.1%) were Wnt1-positive (Table 2). No significant difference in the Wnt1 status was observed according to the tumour histology, tumour status, nodal status, pathological stage or tumour differentiation.

3.2. The β-catenin expression in relation to the Wnt1 status

The intratumoural β-catenin expression exhibited four staining patterns (Fig. 1B and H).[13] Amongst 106 Wnt1-positive tumours, 8 carcinomas (7.5%) had a membranous pattern, 21 carcinomas (19.8%) had a membranous-cytoplasmic pattern, 37 carcinomas (34.9%) had a cytoplasmic pattern and 40 carcinomas (37.7%) had a cytoplasmic-nuclear pattern. Amongst 110 Wnt1-negative carcinomas, 37 carcinomas (33.6%) had a membranous pattern, 45 carcinomas (40.9%) had a membranous-cytoplasmic pattern, 11 carcinomas (10.0%) had a cytoplasmic pattern and 17 carcinomas (15.5%) had a cytoplasmic-nuclear pattern. The ratio of tumours with a cytoplasmic-nuclear pattern or a cytoplasmic pattern was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (72.6% *versus* 25.5%; $p < 0.0001$).

3.3. The c-Myc expression in relation to the Wnt1 status

In order to investigate the biological functions of the Wnt1 expression in NSCLC, we studied four tumour-associated Wnt1-targets such as c-Myc, Cyclin D1, VEGF-A and MMP-7. Regarding the intratumoural c-Myc expression, the percentage of c-Myc-positive tumour cells varied greatly amongst the 216 NSCLCs (median, 39.0%; mean ± SD, 39.5 ± 28.8%; Fig. 1C). Furthermore, the percentage of Wnt1-positive tumour cells significantly correlated with the percentage of c-Myc-positive tumour cells ($r = 0.376$; $p < 0.0001$; Fig. 2A). The percentage of c-Myc-positive tumour cells was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (50.2 ± 26.8% *versus* 29.2 ± 27.0%; $p < 0.0001$; Fig. 2B). Regarding the clinical significance of c-Myc status, the percentage of c-Myc-positive tumour cells significantly correlated with the Ki-67 proliferation index ($r = 0.328$; $p < 0.0001$).

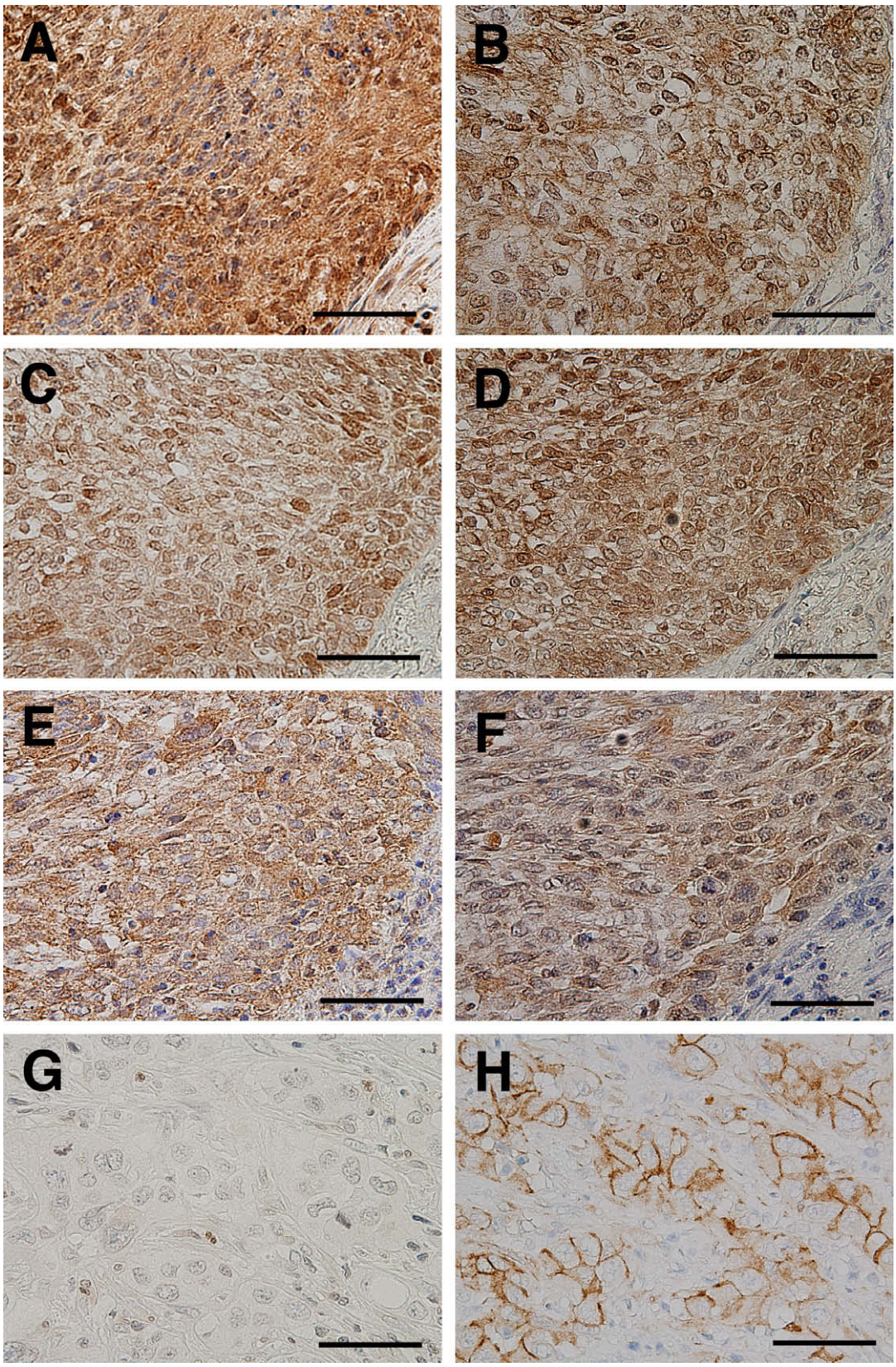

Fig. 1 – Immunostaining of lung cancers. A carcinoma with the positive expression of Wnt1 (A), cytoplasmic-nuclear expression of β-catenin (B), positive expression of c-Myc (C), positive expression of Cyclin D1 (D), positive expression of VEGF-A (E) and positive expression of MMP-7 (F). A carcinoma with negative expression of Wnt1 (G) and membranous expression of β-catenin (H). Bar, 50 μm.

Table 2 – Distribution of Wnt1 status in NSCLC patients according to clinicopathological characteristics

Characteristics	Number of patients	Wnt1 status		*p*-Value
		Negative	Positive	
Smoking				
Non-smoker	75	35	40	0.3611
Smoker	141	75	66	
Tumour status				
T1	87	45	42	0.3212
T2	72	39	33	
T3	18	11	7	
T4	39	15	24	
Nodal status				
N0	153	81	72	0.3559
N1, N2, N3	63	29	34	
Pathological stage				
I	123	65	58	0.3294
II	27	16	11	
III	66	29	37	
Differentiation				
Well	75	44	31	0.2221
Moderately	78	38	40	
Poorly	63	28	35	
Histology				
Adenocarcinoma	123	67	56	0.0832
Squamous cell carcinoma	83	35	48	
Large cell carcinoma	10	8	2	
Total number of patients	216	110	106	

3.4. *The Cyclin D1 expression in relation to the Wnt1 status*

Regarding the intratumoural Cyclin D1 expression, the percentage of Cyclin D1-positive tumour cells varied greatly amongst the 216 NSCLCs (median, 38.5%; mean ± SD, 39.9 ± 26.2%; Fig. 1D). The percentage of Wnt1-positive tumour cells also significantly correlated with the percentage of Cyclin D1-positive tumour cells ($r = 0.410$; $p < 0.0001$; Fig. 2C). The percentage of Cyclin D1-positive tumour cells was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (50.3 ± 26.3% *versus* 29.8 ± 22.0%; $p < 0.0001$; Fig. 2D). Regarding the clinical significance of Cyclin D1 status, the percentage of Cyclin D1-positive tumour cells significantly correlated with the Ki-67 proliferation index ($r = 0.265$; $p < 0.0001$).

3.5. *The VEGF-A expression in relation to the Wnt1 status*

Regarding the intratumoural VEGF-A expression, the percentage of VEGF-A-positive tumour cells varied greatly amongst NSCLCs (median, 33.0%; mean ± SD, 35.5 ± 29.8%; Fig. 1E). The percentage of Wnt1-positive tumour cells significantly correlated with the percentage of VEGF-A-positive tumour cells ($r = 0.164$, $p = 0.0160$). The percentage of VEGF-A-positive tumour cells was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (40.7 ± 31.8% *versus* 30.5 ± 27.0%; $p = 0.0119$; Fig. 2E). Regarding the clinical significance of VEGF-A status, the percentage of VEGF-A-positive tumour cells significantly correlated with the IMD ($r = 0.136$; $p = 0.0452$).

3.6. *The MMP-7 expression in relation to the Wnt1 status*

The percentage of MMP-7-positive tumour cells also varied greatly amongst the 216 NSCLCs (median, 48.0%; mean ± SD, 45.9 ± 30.2%; Fig. 1F). The percentage of Wnt1-positive tumour cells significantly correlated with the percentage of MMP-7-positive tumour cells ($r = 0.383$; $p < 0.0001$). The percentage of MMP-7-positive tumour cells was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (56.7 ± 27.5% *versus* 35.7 ± 29.3%; $p < 0.0001$; Fig. 2F).

3.7. *The clinical significance of the intratumoural Wnt1 status in NSCLC*

Regarding tumour proliferation, the percentage of Wnt1-positive tumour cells significantly correlated with the Ki-67 proliferation index ($r = 0.191$; $p = 0.0048$). The Ki-67 proliferation index was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (53.2 ± 29.5% *versus* 38.8 ± 28.4%; $p = 0.0003$; Fig. 2G).

Concerning tumour angiogenesis, the percentage of Wnt1-positive tumour cells also significantly correlated with the IMD ($r = 0.151$; $p = 0.0267$). The IMD was significantly higher in Wnt1-positive tumours than in Wnt1-negative tumours (113.1 ± 66.3 *versus* 96.8 ± 47.8; $p = 0.0383$; Fig. 2H).

Regarding the survival, the 5-year survival was 42.6% in patients with Wnt1-positive NSCLCs and 66.5% in patients with Wnt1-negative NSCLCs. The overall survival was significantly lower in patients with Wnt1-positive NSCLCs than in patients with Wnt1-negative NSCLCs ($p = 0.0013$; Fig. 3). A

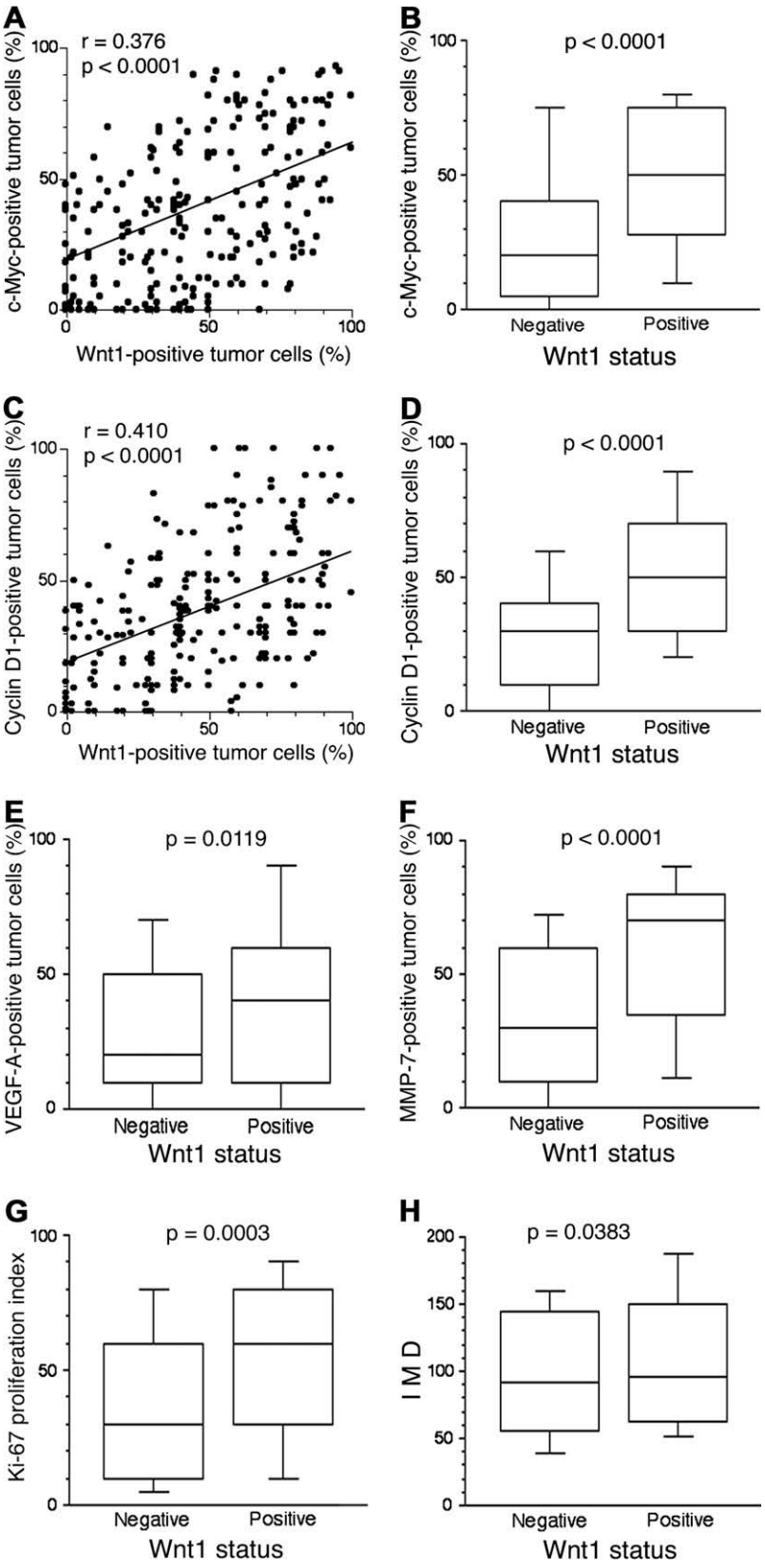

Fig. 2 – (A) The relationship between Wnt1 expression and c-Myc expression, (B) c-Myc expression in relation to the Wnt1 status, (C) The relationship between Wnt1 expression and Cyclin D1 expression, (D) cyclin D1 expression in relation to the Wnt1 status, (E) VEGF-A expression in relation to the Wnt1 status, (F) MMP-7 expression in relation to the Wnt1 status, (G) Ki-67 proliferation index in relation to the Wnt1 status and (H) IMD in relation to the Wnt1 status.

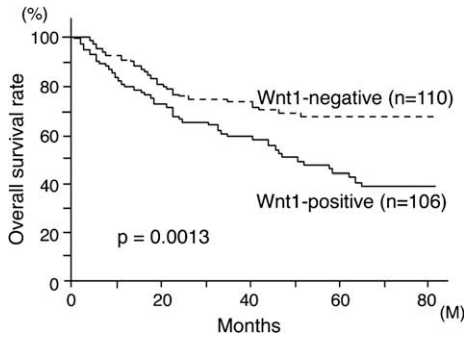

Fig. 3 – Overall survival of NSCLC patients in relation to the Wnt1 status.

multivariate analysis demonstrated that the Wnt1 status (hazard ratio 1.784; $p = 0.0127$), the tumour status (hazard ratio 1.280; $p = 0.0113$) and the nodal status (hazard ratio 1.479; $p = 0.0013$) were significant prognostic factors for NSCLC patients (Table 3).

4. Discussion

The *Wnt* genes encode secreted proteins with multi-directional biological functions *via* autocrine or paracrine routes.[2] They are involved in the regulation of a wide variety of normal and pathological processes, including tumourigenesis.[2,3] Amongst them, Wnt1 is one of the novel members stimulating the canonical Wnt/β-catenin signalling pathway.[5] The activation of the canonical Wnt signalling pathway leads to the stabilisation of β-catenin, subsequently regulating the transcription of many Wnt-target genes with TCF/LEF1 motifs.[6] The Wnt-target genes include various molecules associated with tumourigenesis.[7–10] Therefore, the Wnt1 overexpression could affect the tumour biology during tumour progression.

This clinical study in NSCLC has demonstrated that the intratumoural Wnt1 expression correlates with various tumour-associated Wnt-targets, including c-Myc,[7] Cyclin D1,[8] VEGF-A[9] and MMP-7.[10] As a result, the intratumoural Wnt1 expression was associated with the tumour proliferation, angiogenesis and a poor prognosis in NSCLC patients.

At first, β-catenin is a pivotal component of the canonical Wnt signalling pathway and E-cadherin-associated homotypic cell adhesion.[15] The β-catenin expression is membranous in normal epithelium. In contrast, β-catenin demonstrated four expression patterns in tumour cells. The β-catenin expression in the cytoplasm and/or nuclear could be considered to be an indication of its aberrant expression.[13] This study demonstrated that the aberrant expression of β-catenin was higher in Wnt1-positive NSCLCs than in Wnt1-negative NSCLCs. Although 28 Wnt1-negative carcinomas (12.8%) had the aberrant expression of β-catenin in this study,

Table 3 – Multivariate regression analysis in predicting the overall survival of NSCLC patients

Variables	Assigned score	Hazard ratio	95% CI	*p*-Value
Wnt1 status				
Negative	0	1.784	(1.132–2.813)	0.0127
Positive	1			
Tumour status				
T1	1	1.280	(1.058–1.550)	0.0113
T2	2			
T3	3			
T4	4			
Nodal status				
N0	0	1.479	(1.166–1.876)	0.0013
N1	1			
N2	2			
N3	3			
Age				
<60	0	1.003	(0.597–1.687)	0.9897
⩾60	1			
Gender				
Female	0	1.815	(0.908–3.627)	0.0915
Male	1			
Smoking				
Non-smoker	0	0.798	(0.422–1.507)	0.4861
Smoker	1			
Differentiation				
Well	0	1.195	(0.890–1.604)	0.2355
Moderately	1			
Poorly	2			

Abbreviation: CI, confidence interval.

this might be partly due to other members of the Wnt family, such as Wnt2b.

Regarding targets of the canonical Wnt/β-catenin signalling pathway, the present study demonstrated the intratumoural Wnt1 expression to correlate with the expression of a transcription factor c-Myc.[7] c-Myc is involved in growth control and cell cycle progression by stimulating and repressing the expression of cell cycle regulators.[16] Therefore, c-Myc has pro-mutagenic effects in cancer cell lines. In fact, previous clinical studies have revealed that the c-Myc overexpression is associated with the malignant phenotype in various human cancers.[17,18] This clinical study also demonstrated that the intratumoural c-Myc expression correlated with the tumour proliferation of NSCLC patients. Furthermore, amongst NSCLC patients we studied, the intratumoural c-Myc expression was significantly associated with the patient survival (data not shown).

Cyclin D1 is another target of the canonical Wnt/β-catenin signalling pathway.[8] This study revealed the intratumoural Wnt1 expression to correlate with the Cyclin D1 expression in NSCLCs. Although the *Cyclin D1* gene is frequently amplified in lung carcinomas,[19] this study identified another mechanism of the Cyclin D1 overexpression in NSCLC. Cyclin D1 regulates the G1-to-S phase transition.[20] In fact, its overexpression has been reported to be associated with the tumour proliferation,[21] as also observed in this study.

On the other hand, angiogenesis is essential for tumour growth and metastasis. VEGF-A is a potent and widely distributed angiogenic peptide.[22] Many clinical studies revealed the intratumoural VEGF-A expression to be associated with the tumour angiogenesis and a poor prognosis in cancer patients.[1,23] In fact, amongst NSCLC patients we studied, the intratumoural VEGF-A expression was significantly associated with the patient survival (data not shown). In addition, Bevacizumab (Avastin; Genentech, Inc, South San Francisco, CA), a recombinant humanised version of the murine antihuman VEGF-A monoclonal antibody, has been developed for the clinical treatment of cancer patients, including NSCLC patients.[24] The VEGF-A is also a target of the canonical Wnt/β-catenin signalling pathway.[9] This study revealed that the Wnt1 expression correlated with the intratumoural VEGF-A expression.

MMP-7 is also one of the targets of the canonical Wnt/β-catenin signalling pathway.[10] This study demonstrated the intratumoural Wnt1 expression to correlate with the MMP-7 expression in NSCLCs. Although the biological functions of MMP-7 are still not clearly understood, previous clinical studies have shown an overexpression of MMP-7 to be associated with an aggressive phenotype in many human cancers.[14,25,26] MMP-7 has broad substrate specificity against the components of the extracellular matrix.[27] Furthermore, MMP-7 plays an important role in the ectodomain shedding of cell-surface molecules, such as E-cadherin.[28] Therefore, MMP-7 is considered to be involved in the regulation of these bioactive substances.

In conclusion, this study evaluating NSCLC indicated that the intratumoural Wnt1 expression affects both tumour proliferation and angiogenesis through the induction of its targets such as c-Myc, Cyclin D1, VEGF-A and MMP-7. Therefore, the Wnt1 overexpression can produce more aggressive malignant tumours during the progression of NSCLCs. Furthermore, the Wnt1 overexpression was an independent prognostic factor for NSCLC patients. To our knowledge, this study is the first comprehensive clinical study demonstrating the clinical significance of the Wnt1 expression in NSCLC. In contrast, no significant difference was observed in the patient survival according to chemotherapy amongst stages II–III NSCLCs in this study. This result might be due to the relatively small number of patients we studied.

The activation of the canonical Wnt/β-catenin signalling pathway has been shown to play an important role in the development of colorectal carcinoma, which is mainly caused by inactivating mutations of the *adenomatous polyposis coli* (APC) gene or by activating mutations of the β-*catenin* gene.[29] However, previous clinical studies have revealed these mutations to be rare in NSCLCs.[13] This study has indicated that the Wnt1 overexpression is a possible mechanism of the activation of the canonical Wnt/β-catenin signalling pathway. Experimental studies demonstrated the Wnt1 expression to be regulated by various molecules, such as NF-kappaB.[30] Therefore, the Wnt1 expression might be secondarily regulated in response to a range of changes in many biological molecules during the tumour progression of NSCLC. Further studies should be performed to clarify the mechanism of the Wnt1 overexpression.

From the results given in this study, the Wnt1 can be a candidate of molecular-target therapy for NSCLC. New strategies, such as the RNA inhibition of Wnt1, may be potentially effective treatments for patients with Wnt1-positive NSCLCs.[31] Because the canonical Wnt1/β-catenin signalling pathway affects the biological functions *via* autocrine or paracrine routes, these Wnt1-inhibiting therapies could have bystander effects on tumour tissues. Further studies should be conducted to develop new treatment modalities for Wnt1-positive tumours.

Conflict of interest statement

None declared.

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