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Original Article

Low expression of FZD1 correlates with poor prognosis in non-small cell lung cancer

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Abstract: Purpose: Frizzled-1 (*FZD1*) is closely associated with development of various tumors, but its expression pattern in lung cancer tissues has not been elucidated enough, including the correlation in clinicopathological features and the prognostic significance of *FZD1* of non-small cell lung cancer (NSCLC) patients. Methods: We evaluated immunohistochemical staining for *FZD1* protein in 152 cases of NSCLC. We correlated the line of differentiation with outcome using Chi-square test and Kaplan-Meier analysis. Results: High-expression of *FZD1* was found in 88/152 (57.9%) of NSCLC patients by immunohistochemical analysis. The expression level of *FZD1* was strongly significantly associated with TNM stage ($P = 0.006$) in NSCLC lesions, but not significantly with other clinicopathological features. On Kaplan-Meier survival curves analysis, low *FZD1* expression had shorter overall survival rate and disease-free survival than those with high *FZD1* ($P = 0.001$ and $P = 0.004$, respectively). Conclusion: These results suggest that low expression of *FZD1* might indicate poor prognosis in NSCLC patients and our observations demonstrate that expression of FZD1 serve as a biomarker of disease progression.

Keywords: Frizzled-1, immunohistochemistry, non-small cell lung cancer, prognosis

Introduction

The incidence and mortality of lung cancer are the highest in the malignant tumor in our country, including non-small cell lung cancer (NSCLC) accounts for about 85%. Most diagnosis for patients with NSCLC is advanced stage [1], so the treatment is given priority to with the systemic treatment such as chemotherapy. In the recent ten years, lung cancer has made great progress on the diagnosis and treatment, but about 80% of lung cancer patients died within a year, only about 5% to 10% of the patients can survive for a long time. The deaths of lung cancer are the first among all kinds of malignant tumors, due to lack of effective method of early lung cancer diagnosis, especially in asymptomatic lung cancer [2]. By studying the pathogenesis of lung cancer, we can find accurate and reliable new molecular biomarkers for lung cancer early diagnosis, which become an urgent need to solve the problem of clinical lung cancer diagnosis and treatment.

Frizzled-1 (*FZD1*), Wnt ligand receptor, belongs to Frizzled family and plays a pivotal role in the regulation of embryonic development, cellular differentiation and proliferation as well as apoptosis. *FZD1* still mediates males HCV-infected hepatic fibrosis, idiopathic pulmonary fibrosis (IPF), and the inflammatory caused by microbial. The current studies demonstrate that *FZD1* is closely associated with development of various tumors, such as migration, invasion, chemotherapeutic drug resistance, poor clinical prognosis and so on [3-6]. Overexpression of *FZD1* has been observed in thyroid [7], breast [8], colon [9], pancreatic [10], gallbladder [11], ovarian [12] and prostate [13]. Furthermore, *FZD1* promotes migration and invasion of pancreatic carcinoma [10], leading to chemotherapeutic multidrug resistance of small cell lung cancer [14], breast [8], pancreatic [10], ovarian [12] and neuroblastoma [15], also involving with a poor prognosis in gallbladder carcinoma [11]. However, the function of *FZD1* whether promoting or suppressing tumors diversified,

Table 1. Clinicopathologic characteristics of patients samples and expression of FZD1

Characteristics	n (%)
Gender	
Male	106 (69.7)
Female	46 (30.3)
Age (years)	
<60	62 (40.8)
≥60	90 (59.2)
TNM stage	
I+II	72 (47.4)
III+IV	80 (52.6)
Pathology classification	
Adenocarcinoma	85 (55.9)
Squamous cell carcinoma	53 (34.9)
Others	14 (9.2)
Histological differentiation	
Low	93 (61.2)
Moderate and high	59 (38.8)
FZD1 expression	
Low	64 (42.1)
High	88 (57.9)

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma.

migration, invasion, and the occurrence of acquired drug resistance have not been clearly defined. Limited reports have been made on *FZD1* expression in NSCLC. In this context, *FZD1* has been shown to play an important role in the development of NSCLC. However, its prognostic value is not clear. The goal of our study was to explore the association between *FZD1* expression and NSCLC and investigate the prognostic value of *FZD1* in NSCLC. Therefore, to improve the outcomes of patients with NSCLC, *FZD1* as early and highly accurate diagnostic biomarkers should be identified to determine the underlying lung cancer biology in patients.

Materials and methods

Patients and tissue sample collection

We included in this retrospective study 152 NSCLC patients who were histopathologically diagnosed between January 2005 and December 2013 at the Affiliated Hospital of Guangdong Medical College in China. Hematoxylin and eosin (H&E)-stained slides were reviewed by two pathologists who were blinded to the clinical parameters. Patients with complete clinical data who underwent any form of preoperative chemotherapy and/or radiation therapy were excluded. None of the patients enrolled in this study suffered from other cancers. Tumor-node-metastasis (TNM) classification was determined by UICC/AJCC (7th edition) for the lung [16]. Overall survival (OS) was defined as the time from the first day of diagnosis to the date of death or the date when patients were last known to be alive. Disease-specific survival (DSS) was defined as the time from the first day of diagnosis to death caused by NSCLC. The patients were followed up via telephone calls or re-examination of their records by our hospital follow-up group. The remaining clinical and pathological features are shown in **Table 1**. This study protocol was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical College. Written informed consent was obtained from all subjects involved in this study.

Immunohistochemistry (IHC)

Formalin-fixed, paraffin-embedded tissue blocks from the most representative histologic sections were retrieved from the archives. Antigen retrieval was performed by incubating the tissue slides in 0.01 M citric acid buffer at 100°C for 10 minutes. After blocking with 3% H_2O_2 and 5% fetal bovine serum, the slides were incubated overnight at 4°C with rabbit anti-human *FZD1* antibody (1:200 dilution, sc-130758, Santa Cruz, USA). The slides were then reacted with polymer-horseradish peroxidase reagent. The peroxidase activity was visualized with diaminobenzidine tetrahydroxy chloride solution. The sections were counterstained with hematoxylin. Dark brown cytoplasmic staining of at least 1% tumor cells was defined as positive, and no staining or less than 1% cells stained was defined as negative. As a negative control, we replaced the primary antibody with 5% fetal bovine serum. *FZD1* positivity was graded based on the percentage of tumor cells with positive staining, including strong (score 3+; ≥50%), moderate (2+; 10%-49%), and weak (1+; 1%-9%). The cytoplasm and membrane staining intensity of the tumor cells was scored as follows: 0, no staining; 1, light yellow staining; 2, yellow staining; and 3, brown staining [17, 18]. The staining index was calculated as follows: staining index = staining intensity ×

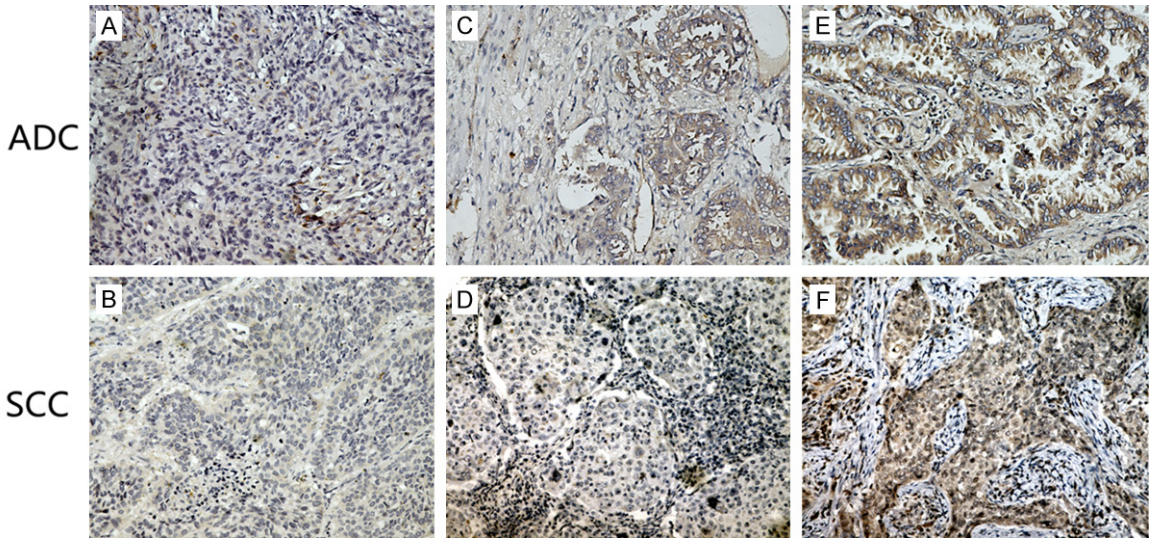

Figure 1. Immunohistochemical expression in the cytoplasm and membrane of *FZD1* in NSCLC tissues. Negative staining of *FZD1* was observed in tumor tissues (A and B). Weak staining of *FZD1* was observed in tumor tissues (C and D). Strong staining of *FZD1* in NSCLC samples was observed in tumor tissues (E and F). All images original magnification ×200 and H&E stained. Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Table 2. Correlation between the clinicopathologic characteristics and expression of FZD1

Clinnicopathological variable	n (%)	FZD1 expression		χ^2	*P*
		Low (%)	High (%)		
Gender				0.017	0.895
Male	106 (69.7)	45 (29.6)	61 (40.1)		
Female	46 (30.3)	19 (12.5)	27 (17.8)		
Age (years)				0.089	0.765
<60	62 (40.8)	27 (17.8)	35 (23.0)		
≥60	90 (59.2)	37 (24.3)	53 (34.9)		
TNM stage				7.486	0.006
I+II	72 (47.4)	22 (14.5)	50 (32.9)		
III+IV	80 (52.6)	42 (27.6)	38 (25.0)		
Pathology classification					
Adenocarcinoma	85 (55.9)	33 (21.7)	52 (34.2)		
Squamous carcinom	53 (34.9)	25 (16.5)	28 (18.4)	0.936	0.626
Others	14 (9.2)	6 (3.9)	8 (5.3)		
Histological differentiation				0.081	0.777
Low	93 (61.2)	40 (26.3)	53 (34.9)		
Moderate and high	59 (38.8)	24 (15.8)	35 (23.0)		

Abbreviations: ADC, adenocarcinoma; SCC, squamous cell carcinoma.

proportion of positively stained tumor cells. By using this method of assessment, we evaluated *FZD1* expression in NSCLC based on the staining index (scored as 0, 1, 2, 3, 4, 6, or 9). For statistical analysis, final staining scores of 0 to 4 and 6 to 9 were considered as low and high expressions, respectively.

Statistical analysis of data

The data analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test was used to analyze the relationship between the levels of *FZD1* expression and clinicopathological characteristics. Survival

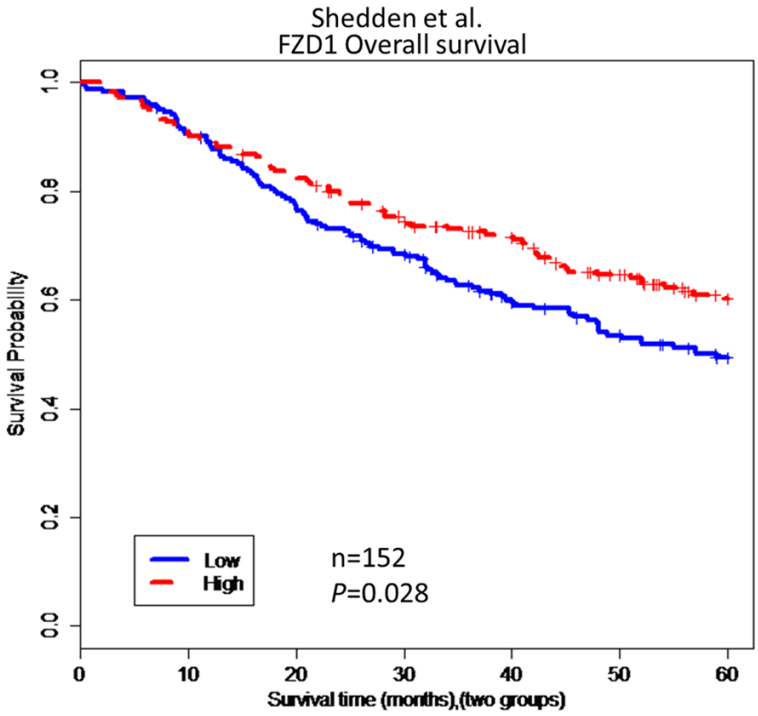

Figure 2. Kaplan-Meier survival analysis of survival time according to the *FZD1* transcript levels as measured using Affymetrix oligonucleotide microarray datasets by Shedden *et al*. [20].

curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox proportional hazards method was used to analyze the relationship between the variables and patient survival time. A two-sided *P* value of less than 0.05 was considered statistically significant.

Results

Expression of FZD1 in NSCLC tissues by IHC analysis

We first examined the *FZD1* expression in 152 cases of NSCLC tissues according to the IHC analysis. Representative staining is shown in **Figure 1**. 57.9% (88/152) cases were high expression of *FZD1*, whereas 42.1% (64/152) were low expression of *FZD1* in the cytoplasm and membrane localization (**Table 1**).

FZD1 expression and clinical characteristics

This cohort included 152 NSCLC cases in total. Among these 152 NSCLC patients, 106 were male and 46 were female. Subjects had an average age of 62.83±10.22 years old (ranging from 27 years old to 84 years old). From the **Table 1**, we could see the clinicopathological features are summarized. To further research the function of *FZD1* in NSCLC tissues, we studied the correlation between *FZD1* protein expression and variety of clinicopathological features of NSCLC. A statistically significant correlation was observed between *FZD1* expression level and TNM stage in NSCLC lesions (P = 0.006) (**Table 2**). As shown in **Table 2**, stratified by TNM stage, there were 72 cases of I+II stage, 14.5% (22/152) cases showed a low expression of *FZD1*, whereas 27.6% (42/152) of the cases comprised the III+IV stage. There was no correlation between *FZD1* expression with other clinical parameters, such as patient gender, age, pathology classification and histological differentiation [19] (**Table 2**).

FZD1 overexpression predicts poor survival in lung cancer

Kaplan-Meier analysis using transcript data for *FZD1* (**Figure 2**) revealed that it predicted poor patient survival with increased expression of these genes in one independent study. Multivariate Cox model analysis (with gene, age, gender, TNM stage and differentiation in the model) indicated that *FZD1* is also independently associated with patient survival in NSCLC [20].

In our study, to further analyze the prognostic significance of *FZD1* expression in NSCLC, we performed the association between *FZD1* expression levels and patient survival using Kaplan-Meier analysis with log-rank test. In 152 NSCLC cases with prognosis information, by univariate analysis, TNM stage and the different levels of *FZD1* protein expression were also significantly correlated with patient OS (P = 0.001) and DSS (P = 0.004) (**Table 3**). We next determined whether *FZD1* provided the independent prognostic information. We performed a multivariate analysis of *FZD1* protein expres-

Table 3. Summary of univariate log-rank analyses of OS and DSS

Parameters	Category	n	OS			DSS		
			No. of events	Median (month)	*P*	No. of events	Median (month)	*P*
Gender	Male	106	61	68.0	0.565	54	52.0	0.914
	Female	46	24	64.0		26	57.0	
Age (years)	<60	62	30	74.0	0.631	31	49.0	0.796
	≥60	90	55	66.0		49	55.0	
TNM stage	I+II	72	33	94.0	< 0.001	32	73.0	< 0.001
	III+IV	80	52	45.0		48	40.0	
	Adenocarcinoma	85	46	65.0		47	54.0	
Pathology classification	Squamous carcinom	53	30	66.0	0.862	26	50.0	0.710
	Others	14	9	76.0		7	55.0	
Histological differentiation	Low	93	51	63.0	0.713	47	50.0	0.865
	Moderate/high	59	34	77.0		33	60.0	
FZD1 expression	Low	64	39	52.0	0.001	39	42.0	0.004
	High	88	46	82.0		41	71.0	

Abbreviations: OS, overall survival; DSS, disease-specific survival; ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Table 4. Summary of multivariate Cox regression analyses of OS and DSS

	Odds ratio (95% CI)	*P*-value
OS		
TNM stage (I+II vs. III+IV)	4.332 (2.666~7.039)	< 0.001
FZD1 expression (low vs. high)	0.569 (0.366~0.885)	0.012
DSS		
TNM stage (I+II vs. III+IV)	3.151 (1.956~5.074)	< 0.001
FZD1 expression (low vs. high)	0.610 (0.391~0.951)	0.029

Abbreviations: OS, overall survival; DSS, disease-specific survival; ADC, adenocarcinoma; SCC, squamous cell carcinoma.

sion levels adjusted for the same parameters. As the **Table 4** shown, the level of *FZD1* expression was an independent prognostic factor for NSCLC. **Table 4** showed that those with low *FZD1* expression had shorter OS and DSS than those with high *FZD1* ($P = 0.012$ and $P = 0.029$, respectively) in multivariate Cox regression analyses.

The Kaplan-Meier survival curves showed that the low level of *FZD1* protein expression among the NSCLC patients predicted a significantly shorter overall survival rate than those with high *FZD1* expression ($P = 0.001$) (**Figure 3**). Results showed that low *FZD1* protein expression was a significant prognostic factor for poor survival in NSCLC. Thus, our observations demonstrate that expression of FZD1 serve as a biomarker of disease progression.

Discussion

FZD1, Wnt signaling pathways of receptor proteins, its expression was also found in a variety of human tumor tissue. Yu, s, et al. [21] found overexpression of E2F1 can cause gene and protein expression of *FZD1* associated with osteoblast differentiation and mineralization in osteosarcoma cell lines. The expression level of *FZD1* increased in ovarian cancer tissue, while reduced in benign ovarian tumor and normal ovarian tissue in turn [12]. Decreased *FZD1* was observed in gallbladder adenocarcinoma (59.0%), adjacent tissue to gallbladder carcinoma (26.1%), adenoma (20.0%), polyps (13.3%), chronic cholecystitis (11.4%) in succession [11]. Clinical research and analysis results showed that the positive expression of *FZD1* was significantly higher in poorly differentiated adenocarcinoma, lump maximum diameter ≥ 2 cm, lymph node metastasis and invade surrounding tissues cases than that of well-differentiated adenocarcinomas, lump maximum diameter < 2 cm, no lymph node metastasis and no infringement to the surrounding tissues. In addition, in the breast cancer research [8] suggests that *FZD1* increased significantly in breast cancer cell lines than in a normal breast epithelial cell. *FZD1* increased in thyroid follicular carcinoma tissue, and there are also some studies have found that the Wnt5a pathway is

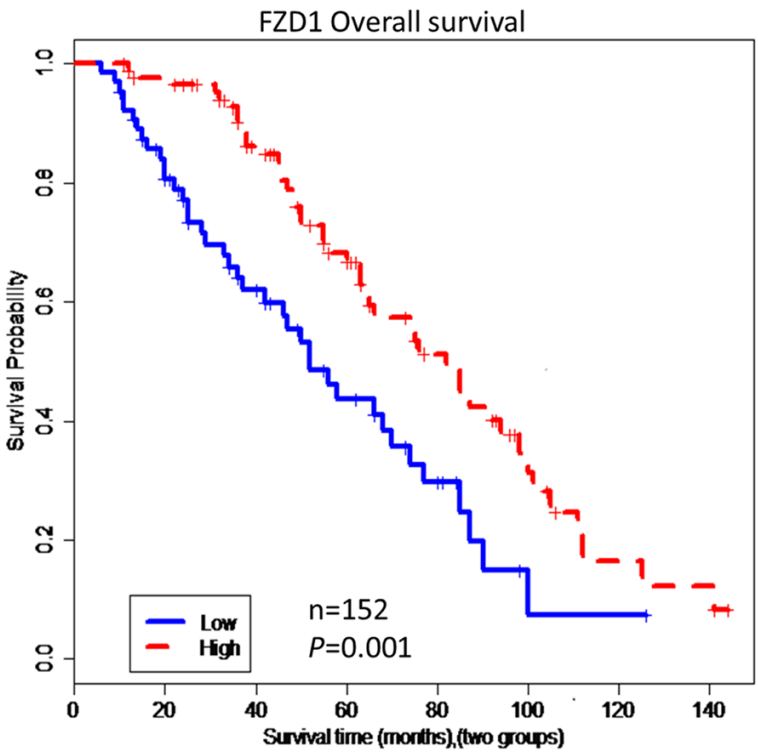

Figure 3. Kaplan-Meier survival analysis of NSCLC patients in OS according to *FZD1* protein expression. It is significant difference in survival rate between the *FZD1*-high expression group and *FZD1*-low group (P = 0.001).

one of the Wnt signaling pathway which take tumor suppressor effect in thyroid cancer [7], but the specific molecular mechanism still remains unclear.

Little is known about *FZD1* in NSCLC patients. The function of *FZD1* in lung cancer tumorigenesis remains unclear and it was a pity that no details were displayed about evaluating the relationship between FZD1 expression and NSCLC clinicopathologic characteristics. In our results, our results likely indicated that *FZD1* plays significant roles in TNM stage in NSCLC, but not relationship with other clinicopathological features. Overexpression of *FZD1* was previously recognized as one of genes involvement in regulating chemoresistance, especially metastasis [22-25]. Li, j, et al. [11] further validated *FZD1* which was a favorable prognostic marker in patients in the gallbladder adenosquamous carcinoma and adenocarcinoma by immunohistochemical and clinical pathological characteristics analysis. However, Shedden et al. [20] showed the *Kaplan-Meier* analysis using transcript data for FZD1 revealed that it predicted poor patient survival with reduced expression of the gene in one independent study. Multivariate Cox model analysis (with gene, age, gender, TNM stage and differentiation in the model) indicated that *FZD1* is also independently associated with patient survival in NSCLC [20]. At the same time, in our results, we observed that the level of *FZD1* protein expression was significantly correlated with the overall survival of NSCLC patients from cumulative survival curves. According to multivariate analyses, we found that low expression of *FZD1* protein was a significant predictor of poor prognosis for NSCLC patients. Our results were consistent with Shedden et al. research results. Therefore, *FZD1* was an important prognostic factor for NSCLC.

To our knowledge, no previous report has been designed to investigate *FZD1* expression and the prognosis in the NSCLC patients. Low expression of *FZD1* demonstrated a poorer overall survival rate than the high expression. *FZD1* expression in NSCLC tissues may be an early event that is related to tumorigenesis. No previous study has been made on this topic. However, the mechanism by which *FZD1* acts on NSCLC patients remains unclear and requires more supporting evidence. Further studies would be needed to prove these findings and to find the role of *FZD1* as a creditable clinical predictor for the outcome of NSCLC the molecular mechanisms.

Conclusion

It's the first time for our data showed that *FZD1* was highly expressed in NSCLC patients and different expression of *FZD1* is associated with TNM stage, which may be the main cause of high mortality among patients with advanced NSCLC cancers. This study results suggest that FZD1 was an important prognostic factor for NSCLC, thereby identifying NSCLC patients that

might benefit from targeting *FZD1* therapy. Finally, it is hoped that the regulation of FZD1 expression in NSCLC may provide benefits in establishing novel therapeutic strategies.

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Disclosure of conflict of interest

None.

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References

[1] Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non small cell lung cancer: epidemiology, risk fctors, treatment, and survivorship. Mayo Clin Proc 2008; 83: 584-594.

[2] Wu X, Piper-Hunter MG, Crawford M, Nuovo GJ, Marsh CB, Otterson GA, Nana-Sinkam SP. MicroRNA in the pathogenesis of Lung Cancer. J Thorac Oncol 2009; 4: 1028-1034.

[3] Minami Y, Oishi I, Endo M, Nishita M. Ror-family receptor tyrosine kinases in noncanonical Wnt signaling: their implications in developmental morphogenesis and human diseases. Dev Dyn 2010; 239: 1-15.

[4] Anastas JN and Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013; 13: 11-26.

[5] Logan CY and Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20: 781-810.

[6] Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006; 127: 469-80.

[7] Kremenevskaja N, von Wasielewski R, Rao AS, Schöfl C, Andersson T, Brabant G. Wnt-5a has tumor suppressor activity in thyroid carcinoma. Oncogene 2005; 24: 2144-54.

[8] Zhang H, Zhang X, Wu X, Li W, Su P, Cheng H, Xiang L, Gao P, Zhou G. Interference of Frizzled 1 (FZD1) reverses multidrug resistance in breast cancer cells through the Wnt/β-catenin pathway. Cancer Lett 2012; 323: 106-13.

[9] Planutis K, Planutiene M, Nguyen AV, Moyer MP, Holcombe RF. Invasive colon cancer, but not non-invasive adenomas induce a gradient effect of Wnt pathway receptor frizzled 1 (Fz1) expression in the tumor microenvironment. J Transl Med 2013; 11: 50.

[10] Kirikoshi H, Katoh M. Expression of WNT7A in human normal tissues and cancer, and regulation of WNT7A and WNT7B in human cancer. Int J Oncol 2002; 21: 895-900.

[11] Li J, Yang ZL, Ren X, Zou Q, Yuan Y, Liang L, Chen M, Chen S. ACE2 and FZD1 are prognosis markers in squamous cell/adenosquamous carcinoma and adenocarcinoma ofgallbladder. J Mol Histol 2014; 45: 47-57.

[12] Badiglian Filho L, Oshima CT, De Oliveira Lima F, De Oliveira Costa H, De Sousa Damião R, Gomes TS, Gonçalves WJ. Canonical and non-canonical Wnt pathway: A comparison among normal ovary, benign ovarian tumor and ovarian cancer. Oncol Rep 2009; 21: 313-20.

[13] Goksel G, Bilir A, Uslu R, Akbulut H, Guven U, Oktem G. WNT1 gene expression alters in heterogeneous population of prostate cancer cells; decreased expression pattern observed in CD133+/CD44+ prostate cancer stem cell spheroids. J BUON 2014; 19: 207-14.

[14] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013; 63: 11-30.

[15] Flahaut M, Meier R, Coulon A, Nardou KA, Niggli FK, Martinet D, Beckmann JS, Joseph JM, Mühlethaler-Mottet A, Gross N. The Wnt receptor FZD1 mediates chemoresistance in neuroblastoma through activation of the Wnt/beta-catenin pathway. Oncogene 2009; 28: 2245-56.

[16] Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, Postmus PE, Rusch V, Sobin L; International Association for the Study of Lung Cancer International Staging Committee; Participating Institutions. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. J Thorac Oncol 2007; 2: 706-14.

[17] Liu W, Zhu J, Cao L, Rodgers GP. Expression of hCG-1 is correlated with differentiation of gastric carcinoma. Histopathology 2007; 51: 157-65.

[18] Besson D, Pavageau AH, Valo I, Bourreau A, Bélanger A, Eymerit-Morin C, Moulière A, Chassevent A, Boisdron-Celle M, Morel A, Solassol J, Campone M, Gamelin E, Barré B, Coqueret O, Guette C. A quantitative proteomic approach

of the different stages of colorectal cancer establishes FZD1 as a new nonmetastatic tumor marker. Mol Cell Proteomics 2011; 10: M111,009712.

[19] Gao Y, Yang M, Jiang Z, Woda BA, Mercurio AM, Qin J, Huang X, Zhang F. IMP3 expression is associated with poor outcome and epigenetic deregulation in intrahepatic cholangiocarcinoma. Hum Pathol 2014; 45: 1184-91.

[20] Director's Challenge Consortium for the Molecular Classification of Lung Adenocarcinoma, Shedden K, Taylor JM, Enkemann SA, Tsao MS, Yeatman TJ, Gerald WL, Eschrich S, Jurisica I, Giordano TJ, Misek DE, Chang AC, Zhu CQ, Strumpf D, Hanash S, Shepherd FA, Ding K, Seymour L, Naoki K, Pennell N, Weir B, Verhaak R, Ladd-Acosta C, Golub T, Gruidl M, Sharma A, Szoke J, Zakowski M, Rusch V, Kris M, Viale A, Motoi N, Travis W, Conley B, Seshan VE, Meyerson M, Kuick R, Dobbin KK, Lively T, Jacobson JW, Beer DG. Gene expression-based survival prediction in lung adenocarcinoma: a multi-site, blinded validation study. Nat Med 2008; 14: 822-827.

[21] Yu S, Yerges-Armstrong LM, Chu Y, Zmuda JM, Zhang Y. E2F1 effects on osteoblast differentiation and mineralization are mediated through up-regulation of frizzled-1. Bone 2013; 56: 234-41.

[22] Ulivieri A, Lavra L, Dominici R, Giacomelli L, Brunetti E, Sciacca L, Trovato M, Barresi G, Foukakis T, Jia-Jing L, Larsson C, Bartolazzi A, Sciacchitano S. Frizzled-1 is down-regulated in follicular thyroid tumours and modulates growth and invasiveness. J Pathol 2008; 215: 87-96.

[23] Zhang H, Zhang X, Wu X, Li W, Su P, Cheng H, Xiang L, Gao P, Zhou G. Interference of Frizzled 1 (FZD1) reverses multidrug resistance in breast cancer cells through the Wnt/beta-catenin pathway. Cancer Lett 2012; 323: 106-13.

[24] Hung TH, Chen CM, Tseng CP, Shen CJ, Wang HL, Choo KB, Chong KY. FZD1 activates protein kinase C delta-mediated drug-resistance in multidrug-resistant MES-SA/Dx5 cancer cells. Int J Biochem Cell Biol 2014; 53: 55-65.

[25] Li J, Yang ZL, Ren X, Zou Q, Yuan Y, Liang L, Chen M, Chen S. ACE2 and FZD1 are prognosis markers in squamous cell/adenosquamous carcinoma and adenocarcinoma of gallbladder. J Mol Histol 2014; 45: 47-57.