Reverse Regulation Between sFRP 5 and Dkk 1 Gene in Early Stage Lung Adenocancer

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Abstract

Purpose: We aimed to examine the expression levels of the genes of APC (Adenomatous Polyposis Coli) 1, APC 2, Dkk (Dickkopf related protein) 1, Dkk -3, sFRP (Secreted frizzled-related protein) -2, sFRP-4, and sFRP-5 genes which play a role in the Wnt signaling pathway in lung adenocarcinoma and adjacent normal lung tissues, and to evaluate their relationship with clinical-pathological factors.

Materials and methods: Between 2011 and 2018, the expression levels of the relevant genes in formalin-fixed paraffin-embedded tumor and adjacent intact lung tissue samples of 57 patients who were operated for lung adenocarcinoma were determined by Real-time PCR analysis.

Results: The expression levels of the Dkk-1 gene in the tumor tissue, especially in stage I-II, were statistically significantly suppressed compared to normal tissue ($p < 0.025$). Although Dkk-1 gene expression was suppressed in the tumor tissue of patients with early-stage lung adenocarcinoma, the level of expression of the sFRP-5 gene was found to be statistically significantly higher ($p < 0.039$).

Conclusion: In our study, between the sFRP-5 and Dkk-1 genes, known as the extracellular antagonist of the Wnt signaling pathway was found the reverse regulation. sFRP-5 gene was found as having an oncogenic role in adenocarcinoma development. Reverse regulation between these genes in early-stage lung adenocarcinoma may shed light on the mechanisms associated with the development of carcinogenesis. For that reason, clinically, this relationship needs to research in a larger series of pure adenocarcinoma and normal human lung tissues, separated by its stage, for potential therapeutic target or prognostic its significance.

Highlights

- * Reverse regulation of DKK-1 and SFRP-5 gene in lung tissues with adenocarcinoma
- * Reverse regulation of two extracellular Wnt signaling antagonists in early-stage lung adenocarcinoma
- * Oncogenic role of the SFRP-5 gene in lung adenocarcinoma
- * This reverse regulation may shed light on lung adenocarcinoma development mechanisms
- * Potential therapeutic target or prognostic significance of this reverse regulation

Introduction

Lung cancers are one of the most important causes of cancer-related deaths. Mortality rates are high due to the fact that most of the patients with lung cancer are detected at an advanced stage and the prognosis is poor. Despite the early diagnosis, curative surgery, and oncological treatments, the recurrence rate is still high in early-stage tumors [1]. Cancer stem cells (CSC) are reported to be responsible for recurrence, resistance to chemo-radiotherapy, and metastasis [3].

Like somatic stem cells, CSCs are managed by Notch, Hedgehog, Wnt /-catenin, Ras-Raf [2-4]. Among these, the Wnt signaling pathway is still not clearly understood. The Wnt / β -catenin signaling pathway provides the transcription of myc, survivin, fibronectin, livin, Oct-4, Axin, Nanog, Snail, etc. proteins that play an important role in proliferation, cell cycle, and differentiation. The other 2 pathways other than the Wnt / β -catenin signaling pathway are called "signal pathways independent of β -catenin". These pathways have been associated with differentiation, cell polarity, and migration [5-9].

Studies on diseases caused by changes in the Wnt signaling pathway and the molecules involved in this pathway are widely available in the literature. The Wnt signaling pathway plays an important role in the occurrence of many diseases such as colorectal cancer (CRC), schizophrenia, retinal angiogenesis defect, Alzheimer's, tetra amelia, polycystic kidney diseases, leukemia, lung cancer, and osteoporosis, with a different mechanism disorder in each tissue [10-17]. The role of APC genes in Wnt signaling in carcinogenesis has been most prominently described for colorectal cancer, but abnormal Wnt signaling is observed in the presence of many more cancers [13,15,16].

The aim of this study is to examine the expression of APC1, APC2, Dkk1, Dkk3, sFRP2, sFRP4, and sFRP5 transcripts in lung adenocarcinoma tissues and their corresponding normal lung tissues and to evaluate their relationship with clinico-pathological factors. We also aimed to help presenting new information about the role of the Wnt signaling pathway in lung adenocarcinoma development and progression.

Materials And Methods

Patients and Tissue Samples: The study was performed on 57 patients operated for lung adenocarcinoma between 2011-2018 in the thoracic surgery clinic. Our institutional Ethics Committee approved the study. The 7-8 mm sections were taken by the pathologist from the samples of tumoral and intact lung tissue embedded in formalin-fixed paraffin samples were centrifuged with xylene, to purify from paraffin, and the supernatant was discharged. After this process was repeated 2-3 times, pure ethanol was added and centrifuged to remove xylene. The supernatant was discharged, and the sample piece was left to dry for 10-15 minutes. 10% SDS, proteinase K, and proteinase K buffer were added onto the tissue piece and incubated at 55 ° C for 3-3.5 hours.

RNA Extraction: In the next process, using the "RNA extraction with triazole" method, total RNA was obtained [18]. The purity and quantitation determinations of the obtained RNAs were made with a nanodrop spectrophotometer.

Reverse Transcriptase Polymerase Chain Reactions (RT-PCR): For cDNA synthesis; for reverse-transcriptase polymerase chain reaction (RT-PCR), a mixture of Poly (T) 18 primers (table 1), 5xRT buffer, 2 mM dNTP, 200 U / μ L RevertAid Reverse Transcriptase, 40 U / μ L RiboLock RNase Inhibitor and distilled water for a total of 45 μ l has been prepared. 5 μ l of the total RNA sample obtained because of RNA isolation was added to the mixture. Under PCR conditions for 30 minutes at 16 ° C, 30 minutes at 42 ° C, and 5 minutes at 85 ° C, the mRNAs were totally converted into cDNA and the samples were stored at -20 ° C [19]. The expression levels of the relevant transcripts were determined by the Quantitative-Comparative CT ($\Delta\Delta$ CT) method using a Real-Time PCR device (Applied Biosystems™ 7500 Real-Time

PCR System). ACTB was chosen as the endogenous control. By placing 20 µl of Master Mix, Forward and Reverse primers, specific probe, and distilled water mixtures into each well of the 96-well plate, 5 µl of cDNA was applied to the relevant mixtures. Real-time PCR analysis were performed for 2 minutes at 50 ° C in the initial phase, 10 minutes at 95 ° C, and 50 cycles at 95 ° C for 15 seconds, at 60 ° C for 90 seconds during the cycle phase. This procedure was repeated 3 times [18,19].

Statistical Analysis

Paired t-test and Wilcoxon signed-rank tests were used to determine whether there was a statistically significant difference between the gene expressions of the tumor and adjacent normal lung tissues. Summary statistics are expressed as median and mean $\pm$ standard deviation. For the relationship between clinical parameters, Fisher's Exact Test was used. In the analysis, p-value <0.05 was considered statistically significant. All analysis were made using the R statistical program.

Construction and analysis of protein-protein interaction networks

We used the String database (<https://www.String-db.org>) version 9.1 for functional, GO process, and KEGG pathway analysis.

Results Of Study

Fifty-seven patients who were operated with the diagnosis of lung adenocarcinoma between 2011 and 2018 were included in the study. Clinico-pathological characteristics of patients is presented in Supplementary Table 1. The mean age of the patients was 63.18 ± 8.28 (min = 47, max = 77). Twenty-five (56.1%) of these patients were below 65 years of age and 32 (43.9%) were over 65 years old. The number of female patients was 22 (38.6%) and the number of male patients was 35 (61.4%). There were 33 (58%) patients with right lung involvement and 24 (42%) patients with left involvement. Clinical staging was done with PET-CT in the preoperative period. Lobectomy was performed in 47 (82.5%) of the patients (Table 2). Minimum lymph node dissections at N2 (n=3) and N1 (n=3) levels were performed in the patients who underwent curative surgical treatment. Forty (70.2%) of the patients were at stage I according to the pathology results (Table 3). Histopathological, 28 (49.1%) of the adenocarcinoma subtypes were of the mixed type (Table 4).

Again, in 8 (14%) patients the tumor was well-differentiated, 49 (86%) were moderately-poorly differentiated. Postoperative patients were followed up for an average of 43 (0-98) months. The average disease-free survival time was 39.4 months. Recurrence was detected in 14 (24.6%) patients during follow-up. Eleven (19.3%) patients died. A second primary tumor was detected in eight (14%) patients during follow-up. The expression of APC1, APC2, Dkk-1, Dkk-3, sFRP-2, sFRP-4, sFRP-5 genes involved in the Wnt signaling pathway in paraffin-embedded tumors and adjacent normal lung tissues of patients were compared. 18 patients did not have normal lung tissues and 3 had against gene expression level was not included in the analysis. A statistically significant difference was found between the Dkk-1 gene expression levels of tumor tissue and neighboring normal lung tissue (p-value = 0.035, Paired t-test) (Figure 1) (Table 5).

In patients with stage I and II, a statistically significant difference was found between tumor tissue and adjacent normal lung tissue Dkk-1 gene expression levels. (p-value = 0.011, paired t-test) (Table 6) (Figure 2).

A statistically significant difference was found between the sFRP-5 gene expression levels of the tumor tissue and the adjacent normal lung tissue in patients with stage I and II (Table 7) (Figure 3). A statistically significant difference was found between tumor tissue and adjacent normal tissue sRFP-5 gene expression levels in patients without lymph node involvement (No) (p-value = 0.011, Wilcoxon signed-rank test) (Table 8) (Figure 4).

Discussion

The Wnt signaling pathway is modulated by extracellular and intracellular control mechanisms. Extracellular antagonists are Dkk, sFRP, Cerebrus, and WIF-1. Inside the cell, Dvl, APC, Axin, CK-1 α , GSK-3 β Wnt play a role in the signal pathway. Especially sFRP and Dkk-1 genes are the most important extracellular antagonists. Dkk-1 and sFRP genes, which play a role in the Wnt pathway, have a tumor-suppressive role, and inhibit the Wnt signaling pathway [21]. Dkk-1 inhibits the canonical Wnt signaling pathway by binding to the Frizzled receptor and LRP 5/6. On the other hand, sFRP genes, bind directly to the Wnt ligand and show an antagonistic effect. sFRP genes inhibit both canonical and non-canonical signaling pathways [21]. The Wnt signaling pathway plays a key role in the development of lung adenocarcinoma [22,23]. In our study, expression levels of APC1, APC2, Dkk-1, Dkk-3, sFRP-2, sFRP-4, sFRP-5 genes involved in the Wnt signaling pathway were evaluated in paraffin-embedded lung adenocarcinoma and adjacent normal lung tissue. The expression levels of the Dkk-1 gene in tumor tissue, especially in stage I-II, were statistically significantly suppressed compared to normal tissue. Although Dkk-1 gene expression was suppressed in tumor tissue of patients with early-stage lung adenocarcinoma, the level of expression of sFRP -5 gene, another extracellular antagonist, was found to be statistically significantly higher. This reverse regulation between Dkk-1 and sFRP -5 gene expressions was striking in our study. In the literature, cross-regulation between the sFRP-1 and Dkk-1 gene has also been associated with multidrug resistance [24].

Another remarkable result is that while the expression level of the Dkk-1 gene in tumor tissues is generally reported to be high in the literature, the opposite of the literature Dkk-1 gene expression was suppressed in our study. Studies of the Wnt signaling pathway in lung cancers have generally been studied in NSCLC cell cultures or adenocancer cell cultures, and studies in human tissues with homogeneous adenocarcinoma tumors are limited in the literature [25–28]. Differences in Dkk-1 gene expression levels have also been reported in NSCLC cell cultures [31]. Dkk-1 gene expression levels in NSCLC tissues of Xiao Yao et al. were in line with the results of our study and were suppressed [29]. Xiao Yao et al. pointed out that this difference in other studies may be ascribed to different sources of tissue samples [29]. The proportion of lung adenocarcinoma tissue is also higher in the authors' studies [29–31]. Our study was homogeneous and consisted of lung adenocarcinoma tissues and mostly stage I. As the stage progresses in lung cancers, the promoter methylation of the Dkk-1 gene increases advanced in other stages compared to the first stage. Overexpression of the Dkk-1 gene in advanced stages leads to

increased cancer invasion and migration [37]. It fulfills this role through the PI3K / Akt signaling pathway [14,37]. For this, BAX and Bcl-2 signal with the AKT-1 pathway by inducing the increase of mRNA in apoptotic gene levels [37]. In our study, the protein-protein relationship of Dkk-1 and sFRP-5 was enriched with the String database [43] (Figure 5). These two proteins with dysregulation have agonistic and antagonistic roles in the Wnt signaling pathway in the GO process analyses [44] (Table 9). Dkk-1 protein is closely related to pro-oncogenes such as Wnt 3a, Wnt 8b, Wnt 8a, Wnt 1 as shown in figure 5. The protein-protein relationship of the Dkk-1 and sFRP-5 genes and the enriched analysis of the KEGG signaling pathway database also revealed their relationship with the mTOR, Hippo signaling pathway [45] (Table 10).

The role of the Dkk-1 gene in human cancer cells is still controversial. In mouse tumor models with advanced NSCLC and in vitro studies, blockade of Dkk-1 gene activity (DKN-01) reduced tumor proliferation and invasion [38]. Again, due to the role of the Dkk-1 gene in bone metabolism, it has been proposed as a biomarker in the early diagnosis of bone metastasis, where high expression levels play a role in bone metastasis in patients with lung cancer [14,30,32]. In our study, high expression levels of sFRP-5, one of the sFRP proteins, which is an extracellular antagonist of the Wnt ligand, were detected in early-stage adenocarcinoma tissues. The sFRP-5 gene is mostly involved in lipoprotein metabolism and plays a protective role in the development of cardiovascular disease [39,40]. There is no study on the relation with Wnt-mediated lung adenocarcinoma development and the sFRP -5 gene in the PubMed database ("*SFRP5 protein, human*" [Supplementary Concept]) AND "*Adenocarcinoma of Lung*" [Mesh]. Especially in the sFRP-5 gene and NSCLC cancer development, sFRP genes are prone to methylation, and methylation of the gene has been reported to be correlated with EGFR mutations. Patients with the methylated sFRP-5 gene have lower disease-free survival rates and higher EGFR_TKI drug resistance than other non-methylated patients [41]. Although sFRP genes are Wnt antagonists, biphasic effects on the Wnt signaling pathway have also been reported. Wnt agonistic effects of sFRP genes by increasing c-JUN-terminal kinase activity through the non-canonical Wnt signaling pathway and inducing an increase in indirect Dkk-1 gene expression levels, increased Dkk gene expression levels in advanced stages have been tried to be explained in the literature [41].

On the other hand, studies by Liang et al. found that sFRP genes play an active role not only outside the cell but also inside the cell, bind B-catenin in the nucleus, and increase the expression of Wnt target genes, showing agonistic effects [42]. Contrary to what is known, the sFRP gene is not antagonistic, but an oncogenic role in the development of adenocarcinoma in the Wnt signaling pathway was also detected at an early stage in our study.

Also, in our study, as an explanation of difference or dysregulation between sFRP 5 and Dkk-1 gene expressions, it was reported that methylation and mRNA expression levels of sFRP 2 and Dkk-2 genes in gastric cancer tissues by Wang H et al. , methylation of these two genes were simultaneous, methylation rates were high, and the mRNA gene expression levels of the Dkk-2 gene were negatively correlated with the methylation rate. These authors argued that this signaling pathway and hypermethylation of genes were the underlying cause of gastric carcinogenesis [46].

In Conclusion

In our study that homogeneous that the expressions were comparing only of in human lung tumors with adenocarcinoma and normal lung tissues, in early-stage lung adenocarcinoma tissue the reverse regulating of the sFRP-5 and Dkk 1 genes known as the extracellular Wnt pathway antagonist were found. sFRP-5 gene was found as having an oncogenic role in early-stage lung adenocarcinoma development. Reverse regulation between these genes in early-stage lung adenocarcinoma may shed light on the mechanisms associated with the development of carcinogenesis. For that reason, clinically, this relationship needs to research in a larger series of pure adenocarcinoma and normal human lung tissues, separated by its stage, for potential therapeutic target or prognostic its significance.

Symbols And Abbreviations Index

sFRP: Secreted frizzled-related protein

Dkk: Dickkopf related protein

FZD: Frizzled receptor

GSK3 β : Glycogen synthase kinase 3 beta

LRP: Lipoprotein receptor-related protein

NSCLC: Non-small cell lung cancer

APC: Adenomatous Polyposis Coli

DVL: Disheveled related protein

CK1 α : casein-kinase 1 α protein

WIF: Wnt inhibitory factor

CSC: Cancer stem cell

EMT: Epithelial-to-mesenchymal transition

JNK: c-Jun N-terminal kinases protein

RYK: Receptor tyrosine kinase protein

ROS: Reactive oxygen species protein

ROR: Receptor tyrosine kinase-like orphan receptor

AXIN: axis inhibition protein

TKI: Tyrosine Kinase Receptor Inhibitor

EGFR: Epidermal Growth Factor Releasing

BAX: BCL2 Associated X

Bcl-2: B-cell lymphoma

KEGG: Kyoto Encyclopedia of Genes and Genomes

Declarations

Ethics approval and consent participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Muğla Sıtkı Koçman University accepted the decision on 13.12.2018 and number 169 of the Ethics Committee of Human Investigations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

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Tables

Table 1. Primer/probe sequences of ACTB, APC1, APC2, Dkk1, Dkk3, sFRP2, sFRP4, and sFRP5 genes to be analyzed by quantitative RT-PCR

Gene Name	Gene ID*	NCBI Reference Sequence Number**	Primer/probe sequence***
ACTB	60	NM_001101.4	F 5'-GGCACCCAGCACAATGAAG-3' R 5'-GCCGATCCACACGGAGTACT-3' Pr 5'-TCAAGATCATTGCTCCTCCTGAGCGC-BHQ-1-3'
APC1	324	NM_022662.2	5'-GAACCTGGAGGTACTTCATGTGAA-3' 5'-GCAAATGTTTGGCCCACTAAA-3' 5'-FAM-AGCGCTTCGTTCAAACAGCAATCCA-BHQ-1-3'
APC2	10297	XM_005259475.2	5'-CATCAGCGAGCGGATGTG-3' 5'-GCAGTTCTCGATGCAGATGGA-3' 5'-FAM-CCTCGAACTCAAGCTGCTGCCGG-BHQ-1-3'
Dkk1	22943	NM_012242.3	F 5'-AATGTATCACACCAAAGGACAAGAAG-3' R 5'-CCAGAAGTGTCTAGCACAAACACAA-3' Pr 5'-FAM-TTTGTCTCCGGTCATCAGACTGTGCC-BHQ-1-3'
Dkk3	27122	NM_015881.5	5'-GTGCAAGCCGACCTTCGT-3' 5'-CTCCATGAAGCTGCCAACTTC-3' 5'-FAM-CCTGCTGCCCAGAGAGGTCCCC-BHQ-1-3'
sFRP2	6423	NM_003013.2	F 5'-TGCTTGAGTGCGACCGTTT-3' R 5'-CAGGAGGTGGTCGCTGCTA-3' Pr 5'-FAM-ACAACGACCTTTGCATCCCCCTCG-BHQ-1-3'
sFRP4	6424	NM_003014.3	5'-GCCGTGCTGCGCTTCTT-3' 5'-TGATAGGGTCGTGCAGGAACT-3' 5'-FAM-CCATGTACGCGCCCATTTGCAC-BHQ-1-3'
sFRP5	6425	NM_003015.3	5'-TGATTGGAGCCCAGAAAAAGA-3' 5'-TGGTGTCTTTCGCTTCAG-3' 5'-FAM-AAGCTGCTCAAGCCGGGCCC-BHQ-1-3'

* <http://www.ncbi.nlm.nih.gov/gene>

** <http://www.ncbi.nlm.nih.gov/RefSeq/>

Table 2: Operative methods

Operations	Number	Ratio (%)	Totally ratio (%)
Lobectomy	47	82.5	82.5
Segmentectomy	3	5.3	87.7
Pneumonectomy	3	5.3	93.0
Wedge Resection	1	1.8	94.7
Bileobectomy	3	5.3	100.0
Total	57	100.0	

Table 3: Distribution of cases according to pathological staging

Stages	Number	Ratio (%)	Totally ratio (%)
Stage I	40	70.2	70.2
Stage II	7	12.3	82.5
Stage III	9	15.8	98.2
Stage IV	1	1.8	100.0
Total	57	100.0	

Table 4: Adenocarcinoma subtypes and rates

Subtype	Number	Ratio (%)	Total ratio
Untyped	12	21.1	21.1
Solid Type	4	7.0	28.1
Acinar Type	10	17.5	45.6
Mixt Type	28	49.1	94.7
Lepidic Type	1	1.8	96.5
Minimal invasive Type	2	3.5	100.0
Total	57	100.0	

Table 5: Comparison of studied gene expression levels in tumor and normal tissue

Gene	Tissue	Sample number	Median	Mean $\pm$ SD	P-value
APC1	Tumor	39	0.78	1.01 $\pm$ 0.76	0.656
	Neighboring normal lung tissue		0.72	1.05 $\pm$ 1.02	
	Difference= Tumor – Normal		0.02	-0.03 $\pm$ 0.48	
APC2	Tumor	38	0.85	2.42 $\pm$ 4.10	0.562
	Neighboring normal lung tissue		0.72	2.24 $\pm$ 3.32	
	Difference= Tumor – Normal		0.02	0.19 $\pm$ 1.98	
DKK-1	Tumor	27	1.19	3.18 $\pm$ 3.96	0.035
	Neighboring normal lung tissue		3.50	5.55 $\pm$ 6.14	
	Difference= Tumor – Normal		-1.36	-2.37 $\pm$ 5.51	
DKK-3	Tumor	33	0.65	1.15 $\pm$ 1.11	0.459
	Neighboring normal lung tissue		0.64	1.20 $\pm$ 1.28	
	Difference= Tumor – Normal		0.02	-0.05 $\pm$ 0.38	
SFRP-2	Tumor	38	0.75	1.32 $\pm$ 1.53	0.373
	Neighboring normal lung tissue		0.65	1.19 $\pm$ 1.38	
	Difference= Tumor – Normal		0.08	0.13 $\pm$ 0.86	
SFRP-4	Tumor	39	0.77	1.23 $\pm$ 0.90	0.761
	Neighboring normal lung tissue		0.75	1.20 $\pm$ 1.21	
	Difference= Tumor – Normal		0.07	0.04 $\pm$ 0.71	
SFRP-5	Tumor	39	0.92	1.87 $\pm$ 2.15	0.331
	Neighboring normal lung tissue		0.68	1.69 $\pm$ 2.31	
	Difference= Tumor - Normal		0.21	0.18 $\pm$ 1.15	

Table 6: Comparison of tumor tissue and neighboring normal lung tissue DKK-1 gene expression levels in early-stage patients

Gene	Tissue	Sample number	Median	Mean $\pm$ SD	P-value
DKK-1	Tumor	23	1.066	2.547 $\pm$ 3.288	0.025
	Neighboring normal lung tissue		3.083	4.945 $\pm$ 5.681	
	Difference= Tumor – Normal		-1.060	-2.398 $\pm$ 4.796	

Table 7: Comparison of SFRP-5 gene expression levels of tumor tissue and neighboring normal lung tissue in early-stage patients

Gene	Tissue	Sample number	Median	Mean $\pm$ SD	P-value
SFRP-5	Tumor	34	0.919	1.516 $\pm$ 1.635	0.039
	Neighboring normal lung tissue		0.641	1.325 $\pm$ 1.802	
	Difference= Tumor - Normal		0.219	0.191 $\pm$ 0.751	

Table 8: Comparison of tumor tissue and neighboring normal tissue SRFP-5 gene expression levels in patients without nodal involvement (No)

Gene	Tissue	Sample number	Median	Mean $\pm$ SD	P-value
SFRP-5	Tumor	28	0.919	1.471 $\pm$ 1.686	0.008
	Neighboring Normal Tissue		0.641	1.166 $\pm$ 1.618	
	Difference= Tumor - Normal		0.298	0.305 $\pm$ 0.614	

Table 9: Analyses table for GO-enriched biological process in which DKK-1 and SFRP-5 genes play a role

Biological Process (GO)			
<i>GO-term</i>	<i>Description</i>	<i>count in gene set false discovery rate</i>	
GO:0016055	Wnt signaling pathway	7 of 303	2.15e-10
GO:0060828	regulation of canonical Wnt signaling pathway	6 of 218	3.35e-09
GO:0009952	anterior/posterior pattern specification	5 of 197	3.02e-07
GO:0060070	canonical Wnt signaling pathway	4 of 93	2.26e-06
GO:1904886	beta-catenin destruction complex disassembly	3 of 21	5.20e-06
GO:0090090	negative regulation of canonical Wnt signaling pat.	4 of 123	5.20e-06
GO:0007369	Gastrulation	4 of 158	1.02e-05
GO:0061317	canonical Wnt signaling pathway involved in cardia	2 of 2	3.78e-05
GO:0044335	canonical Wnt signaling pathway involved in neural..	2 of 2	3.78e-05
GO:0001756	Somitogenesis	3 of 63	6.15e-05
GO:0090245	axis elongation involved in somitogenesis	2 of 4	7.56e-05
GO:0030279	negative regulation of ossification	3 of 71	7.87e-05
GO:0009966	regulation of signal transduction	7 of 3033	9.06e-05
GO:0090244	Wnt signaling pathway involved in somitogenesis	2 of 5	9.20e-05
GO:0044332	Wnt signaling pathway involved in dorsal/ventral a.	2 of 5	9.20e-05
GO:0060021	roof of mouth development	3 of 87	0.00010
GO:0009996	negative regulation of cell fate specification	2 of 6	0.00010
GO:0009953	dorsal/ventral pattern formation	3 of 87	0.00010
GO:0009798	axis specification	3 of 89	0.00010
GO:0090381	regulation of heart induction	2 of 7	0.00011
GO:0042661	regulation of mesodermal cell fate specification	2 of 7	0.00011
GO:0022411	cellular component disassembly	4 of 364	0.00011
GO:0009790	embryo development	5 of 890	0.00011
GO:0032526	response to retinoic acid	3 of 104	0.00012

GO:0051090	regulation of DNA-binding transcription factor acti.	4 of 403	0.00013
GO:1904953	Wnt signaling pathway involved in midbrain dopamin	2 of 10	0.00015
GO:1904338	regulation of dopaminergic neuron differentiation	2 of 10	0.00015
GO:0009653	anatomical structure morphogenesis	6 of 1992	0.00015
GO:0014029	neural crest formation	2 of 11	0.00017
GO:0030177	positive regulation of Wnt signaling pathway	3 of 126	0.00018

(number of nodes: 12)

(number of edges: 64)

(average node degree: 10.7)

(avg. local clustering coefficient: 0.973)

(expected number of edges: 11)

(PPI enrichment p-value:< 1.0e-16)

Table 10: Analyses of KEGG enriched signaling pathway in which DKK-1 and SFRP-5 genes play a role

KEGG Pathways			
<i>pathway</i>	<i>Description</i>	<i>count in gene set</i>	<i>false discovery rate</i>
hsa04310	Wnt signaling pathway	6 of 143	1.84e-11
hsa05226	Gastric cancer	4 of 147	8.78e-07
hsa05225	Hepatocellular carcinoma	4 of 163	8.78e-07
hsa05224	Breast cancer	4 of 147	8.78e-07
hsa04150	mTOR signaling pathway	4 of 148	8.78e-07
hsa05200	Pathways in cancer	4 of 515	4.01 e-05
hsa05217	Basal cell carcinoma	2 of 63	0.00048
hsa04916	Melanogenesis	2 of 98	0.0010
hsa04550	Signaling pathways regulating pluripotency of stem cells	2 of 138	0.0017
hsa04934	Cushing's syndrome	2 of 153	0.0019
hsa04390	Hippo signaling pathway	2 of 152	0.0019
hsa05205	Proteoglycans in cancer	2 of 195	0.0026
hsa05166	HTLV-I infection	2 of 250	0.0038
hsa05165	Human papillomavirus infection	2 of 317	0.0056

Figures

Figure 1

Graphical representation of the comparison of Wnt gene expression levels studied in lung adenocarcinoma and normal lung tissues.

Figure 2

Graphical representation of the comparison of Dkk-1 gene expression levels in early-stage lung adenocarcinoma and normal lung tissues.

Figure 3

Graphical representation of the comparison of sFRP-5 gene expression levels in early-stage lung adenocarcinoma and normal lung tissues.

Figure 4

Graphical representation of the comparison of sRFP-5 gene expression levels in early-stage lung adenocarcinoma and normal lung tissues with No.

Figure 5

Protein-protein relationship of Dkk-1 and sFRP-5 genes enriched with String Database (43)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementaryTable1.docx](#)