*Original Article*
# The correlation between DKK1 expression and CT results in the diagnosis of non-small cell lung cancer

Dianjun Zou, Xiaolong Zhu, Qingxiao Liu, Shujun Cui

*Department of Medical Imaging, The First Affiliated Hospital of Hebei North University, Zhangjiakou, Hebei, China*

**Abstract:** Objective: The aim of this study was to assess the correlation of Dickkopf-related protein 1 (DKK1) level with computed tomography (CT) characteristics in patients with non-small cell lung cancer (NSCLC). Methods: A total of 58 NSCLC patients and 10 healthy subjects treated in The First Affiliated Hospital of Hebei North University hospital from May 2011 to November 2015 were randomly enrolled. The carcinoma tissue and para-carcinoma tissue after operation and CT imaging data were collected. The serum DKK1 level was detected by ELISA and DKK1 mRNA and protein levels were measured via qPCR and immunohistochemistry (IHC). Moreover, the correlation between CT signs and DKK1 expression was analyzed, along with analysis of the relations of DKK1 expression with progression-free survival (PFS) and overall survival (OS). Results: DKK1 concentration in NSCLC patients [(185.2±29.4) pg/mL] was significantly elevated compared to healthy individuals [(105.6±18.5) pg/mL] (P<0.05). The DKK1 mRNA level in NSCLC tissues was 2.65 times higher than in the para-carcinoma tissues (P<0.01). Meanwhile, a significantly higher DKK1 positive rate (67.24%, 39/58) was found in NSCLC tissue compared to that in para-carcinoma tissues (25.86%, 15/58) (P<0.05). The DKK1 protein level in patients with a tumor diameter ≥3 cm, enhancement value ≥20 HU and ground-glass sign was markedly higher than that in patients with a tumor diameter <3 cm, enhancement value <20 HU and no ground-glass signs (P<0.05). According to the survival analysis, the OS of patients with high DKK1 level (median OS: 15.4 months) was statistically shorter than those with low DKK1 level (median OS: 18.34 months) (P=0.0219). Conclusion: DKK1 is abnormally overexpressed in NSCLC tissues, which is related to the CT scanning signs. The detection of DKK1 protein expression in NSCLC tissues can non-invasively facilitate the CT observation, which might guide clinical diagnosis and treatment of NSCLC.

Keywords: Non-small cell lung cancer, DKK1, CT

## Introduction

Lung cancer is a major contributing factor to cancer-related deaths [1]. It includes several histological types, and non-small cell lung cancer (NSCLC) is the most common type [2, 3]. NSCLC treatment is still overall unsatisfactory due to various clinical problems. A large proportion of patients are in advanced stages or have metastatic disease once diagnosed, while only one-third of NSCLC patients can be treated with intervention treatment via radical operation technically and oncologically. Due to high heterogeneity of NSCLC, the prognosis is often poor [4]. Currently, validated biomarkers are urgently needed for clinical screening, which is of great importance for not only early diagnosis

but also the choice of optimal therapeutic intervention.

It has been demonstrated in previous studies that the Wnt signaling pathway regulates the cell proliferation, polarity and migration [5, 6]. The Dickkopf family protein DKK1 encodes a Wnt antagonist and inhibits the Wnt/β-catenin pathway [7]. DKK1 expression is often significantly declined in human colon cancer, gastric cancer and melanoma [8, 9], but it is overexpressed in hepatocellular carcinoma (HCC) and Wilms' tumor [10], indicating that the function of DKK1 may vary from cancer to cancer. However, whether DKK1 level affects NSCLC clinicopathological factors as well as has a correlation with the CT results of NSCLC remain unclear. Our study intends to assess the rela-

tionship of DKK1 expression with NSCLC clinicopathological features.

## Patients and methods

*General data*

The clinical data of 58 NSCLC patients treated in the First Affiliated Hospital of Hebei North University from May 2011 to November 2015 were enrolled. All patients were examined with chest spiral CT. There were 33 males and 25 females aged 38-76 years old (average: 58.18± 8.54 years old). There were 42 cases of lung adenocarcinoma and 16 cases of lung squamous cell carcinoma. Inclusion criteria were: 1) patients meeting *"Diagnostic Criteria for Primary Lung Cancer"*, 2) patients with the Karnofsky performance score (KPS) ≥80 points, 3) patients without severe organ dysfunction, 4) patients without coagulation dysfunction, 5) patients without gastrointestinal tumor, breast cancer and other tumors, and 6) patients who gave their informed consent. Exclusion criteria were: 1) patients with impaired function of the liver, kidney or heart, 2) patients with mental disorders, or 3) patients whose clinical data were incomplete or who were lost to follow-up. Meanwhile, 10 healthy individuals were recruited as the control group and no significant difference regarding the general data, such as age, gender, weight, was present. All participants signed the informed consent and fasting blood was drawn. This study was approved by the Ethics Committee of the First Affiliated Hospital of Hebei North University.

*Detection of DKK1 mRNA expression level via quantitative PCR (qPCR)*

After homogenization of the tissue, the total RNA was isolated (Qiagen, Shanghai, China) for cDNA synthesis, followed by qPCR using the SYBR® Premix Ex Taq™ II kit (Takara): with a total of 45 cycles of amplification under annealing temperature of 55°C. Gene expression was assessed using $2^{-\Delta Ct}$, with GAPDH as a reference. Primer sequences were: DKK1: Forward: 5'-TGGTCCAAGATCTGTAAACCTGTCC-3', Reverse: 5'-CTGGCTTGATGGTGATCTTTCTGTA-3', GAPDH: Forward: 5'-ATTGATGGATGCTAFGA-GTATT-3', Reverse: 5'-AGTCTTCTGGGTGGCAG-TGAT-3'. This experiment was performed in triplicate.

*Detection of level of serum DKK1 in NSCLC patients via ELISA*

Before enrollment, 5 mL whole blood was collected and centrifuged to separate the serum. The standard sample was diluted at 1:50, and the standard curve was plotted. The DKK1 (article No.: P5326, New York, USA) protein level in serum samples was detected using the ELISA kit (RD, USA).

*Detection of DKK1 protein expression via IHC*

The carcinoma tissues and para-carcinoma tissues were prepared into paraffin-embedded blocks and sliced into 4-μm-thick sections followed by being incubated at 65°C for 3-4 h, with deparaffinization and dehydration, for antigen retrieval. After blockage of the peroxidase and sealing, primary antibody (DKK1, Abcam, USA, diluted at 1:300) was added for overnight incubation at 4°C followed by washing and incubation with secondary antibody and subsequent color development using diaminobenzidine (DAB). The positive/total cells >10% indicates a positive expression.

*CT scan*

The 64-slice spiral CT (TOSHIBA, Japan) was performed from the apex of lung to the bilateral costophrenic angle with the following indicators: voltage: 120 kV, current: 180 mA, and slice thickness: 5 mm. The multi-planar reconstruction and three-dimensional reconstruction were performed for lung lesions at an interval of 3 mm, and a 1-mm-thin-slice reconstruction could be conducted if necessary. The iodine allergy test was performed routinely: 60 mL iohexol solution was injected using a high-pressure syringe at an injection rate of 4-5 mL/s. The delayed scan was performed if necessary, to measure the lesion size and CT values. The original data obtained were sent to the background processor for diagnostic analysis using the Aquilion software assistant. The CT examination results of all patients were diagnosed jointly by two physicians.

*Statistical methods*

SPSS 19.0 software was utilized for analyzing data which were displayed as mean ± SD and assessed by Student's *t*-test. Kaplan-Meier
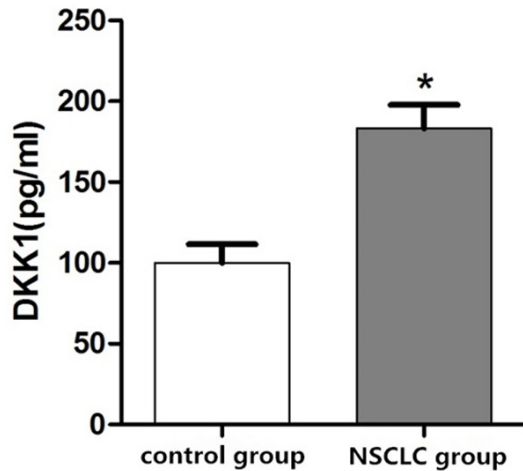
**Figure 1.** Expression level of serum DKK1 in NSCLC patients detected via ELISA. *P<0.05.



**Figure 2.** DKK1 mRNA level in carcinoma tissues and para-carcinoma tissues. **P<0.01.

method assessed the survival. *P<0.05* indicates significance [9].

### Results
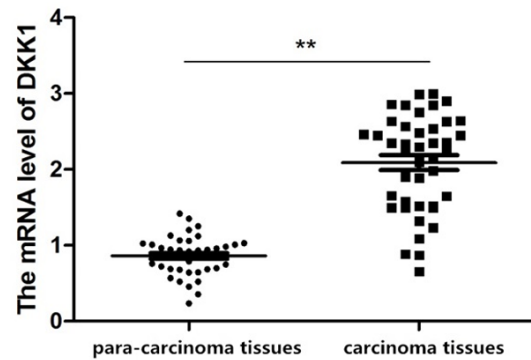
*Level of serum DKK1 in NSCLC patients detected via ELISA*

In NSCLC patients, DKK1 protein concentration was [(185.2±29.4) pg/mL], which was significantly higher than that in the control group [(105.6±18.5) pg/mL] (*P<0.05*) (**Figure 1**).

*qPCR*

The result of qPCR further confirmed that the DKK1 mRNA level in NSCLC tissues was 2.65 times higher than that in the para-carcinoma tissues (*P<0.01*) (**Figure 2**).

*DKK1 protein level in NSCLC tissues and para-carcinoma tissues detected via IHC*

In the cytoplasm of NSCLC tissues, a weakly positive or positive result of DKK1 expression was observed, and the staining displayed a brown yellow or dark brown color in different degrees. In para-carcinoma tissues, the DKK1 expression was weakly negative or negative (**Figure 3**). The positive rate of DKK1 was 67.24% (39/58) in 58 cases of NSCLC tissues and 25.86% (15/58) in 58 cases of para-carcinoma tissues, indicating that there was higher DKK1 protein level in NSCLC than that in para-carcinoma tissues (*P<0.05*).

*Correlation between CT manifestations and serum DKK1 concentration (ng/mL) in lung cancer*

The commonly-used CT imaging signs of lung cancer can determine the presence or absence of lobulated signs, pleural indentation, spicule signs, fluid bronchogram signs, cavity, vocule signs, ground-glass signs, calcification, enlargement of hilar and mediastinal lymph nodes, tumor diameter and enhancement value (**Figure 4**). Our data showed that the DKK1 protein level in 58 NSCLC patients with a tumor diameter ≥3 cm, enhancement value ≥20 HU and ground-glass signs was markedly higher than that in patients with a tumor diameter <3 cm, enhancement value <20 HU and no ground-glass signs (*P<0.05*). No significant differences of other imaging signs were indicated between the two groups (*P>0.05*) (**Table 1**).

*Survival curve*

Kaplan-Meier curve revealed that the OS of patients with high DKK1 level was statistically shorter (median OS: 15.4 months) than patients with low DKK1 level (median OS: 18.34 months) (*P=0.0219<0.05*) (**Figure 5**).

### Discussion

The DKK protein family functions as a Wnt/β-catenin signaling antagonist [11]. It is noted that abnormal Wnt signaling is a major feature of a variety of human cancers and this pathway mediates the biological processes of various tumor cells [12]. The DKK family is composed of DKK1, DKK2, DKK3 and DKK4, all of which jointly express a kind of DKK3-related protein
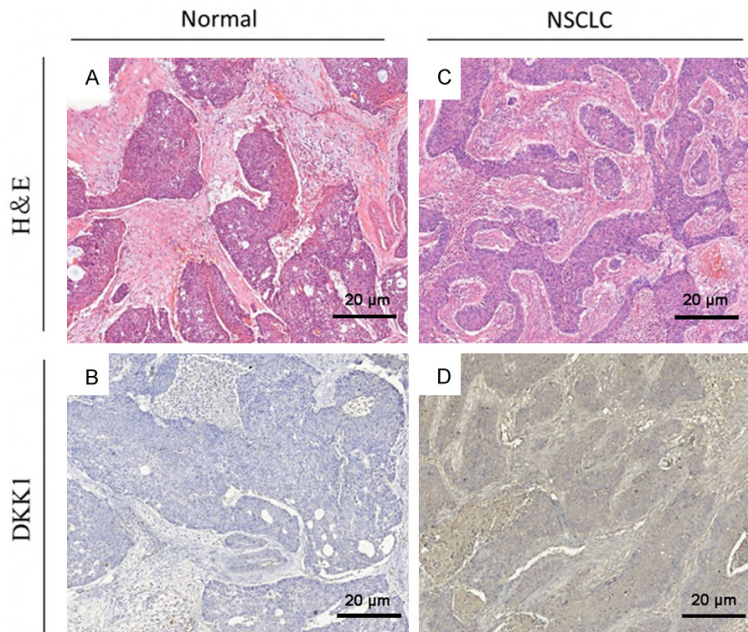
**Figure 3.** DKK1 protein expression in NSCLC tissues and para-carcinoma tissues detected via IHC (scale bar 20 μm).
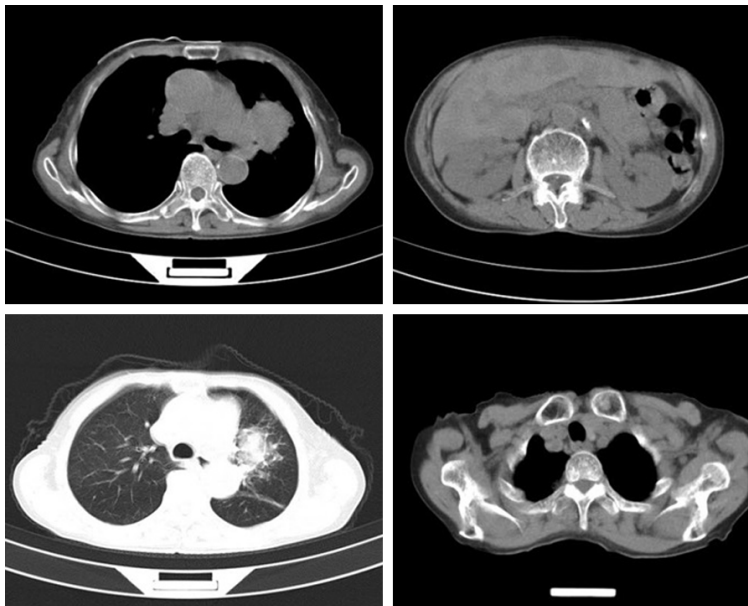


**Figure 4.** CT data of an NSCLC patient. A 52-year-old male patient with NSCLC has left central lung cancer accompanied with mediastinal and left axillary lymph nodes, and multiple intrahepatic metastases.

DKK1 is a kind of secretory protein and its serum level in disease prognosis has been assessed in several cancers [16]. Previous evidence showed significantly elevated DKK1 serum levels in early HCC patients compared to that in patients with cirrhosis, with non-cirrhotic hepatitis B or healthy people (P<0.001). Of note, the OS and PFS of patients with high DKK1 level are lower than patients with a low level (P=0.028, P=0.045) [17]. Recently, Cao HZ et al. found abnormally high DKK1 positive rates in cervical cancer [18]. In addition, DKK1 level is correlated with lymph node metastasis in cervical cancer. Our study found higher DKK1 level in NSCLC patients compared to that in normal individuals, which is in accordance with the above research results.

Moreover, DKK1 at both the mRNA and protein level in NSCLC tissues is significantly higher than that in para-carcinoma tissues. Importantly, the survival time of patients with high DKK1 levels was significantly reduced compared to those with low levels (P=0.0219), indicating that DKK1 is implicated to the occurrence and development of NSCLC, and the high-level DKK1 may be a potential marker for poor prognosis.

CT is widely used for NSCLC diagnosis. CT imaging signs of lung cancer can identify the presence or absence of lobulated signs, pleural indentation, spicule signs, fluid bronchogram signs, cavity, vocule signs, ground-glass signs, calcification, enlargement of hilar and mediastinal lymph nodes, tumor diameter and enhancement value [19]. The tumor size is a manifestation of tumor growth, progression and invasive activity. Archibugi L argued that the period

known as Soggy [13]. DKK1 can also inhibit cell growth and trigger apoptosis [14]. After binding to LRP5/6, DKK1 causes degradation of β-catenin and affects cell proliferation. According to another study, however, DKK1 also serves as an agonist that up-regulates other Wnt signaling pathways [15].

**Table 1.** Correlation between CT manifestations and serum DKK1 concentration (ng/mL) in lung cancer

| CT manifestation | | n | Serum DKK1 concentration (ng/mL) | t | P |
|---|---|---|---|---|---|
| Deep lobulated signs | Yes | 26 | 2.56±0.73 | 0.65 | 0.542 |
| | No | 32 | 1.59±0.21 | | |
| Pleural indentation | Yes | 25 | 3.65±0.79 | | |
| | No | 33 | 1.56±0.78 | | |
| Tumor diameter | ≥3 cm | 36 | 5.36±0.98 | 3.76 | 0.012 |
| | <3 cm | 22 | 2.23±0.53 | | |
| Spicule signs | Yes | 30 | 2.69±1.35 | 0.61 | 0.244 |
| | No | 28 | 1.32±0.95 | | |
| Fluid bronchogram signs | Yes | 9 | 1.33±0.23 | 0.45 | 0.174 |
| | No | 49 | 1.69±0.89 | | |
| Enhancement value | ≥20 HU | 31 | 5.31±0.52 | 2.64 | 0.012 |
| | <20 HU | 27 | 1.74±0.63 | | |
| Cavity | Yes | 5 | 3.65±0.51 | 0.82 | 0.254 |
| | No | 53 | 2.85±0.66 | | |
| Vocule signs | Yes | 10 | 1.52±0.35 | 0.36 | 0.519 |
| | No | 48 | 1.26±0.69 | | |
| Ground-glass signs | Yes | 8 | 2.95±0.35 | 2.54 | 0.015 |
| | No | 50 | 1.15±0.12 | | |
| Calcification | Yes | 1 | 2.36±0.32 | 0.53 | 0.361 |
| | No | 57 | 1.09±0.13 | | |
| Enlargement of hilar and mediastinal lymph nodes | Yes | 33 | 2.74±0.63 | 1.87 | 0.013 |
| | No | 25 | 1.12±0.53 | | |
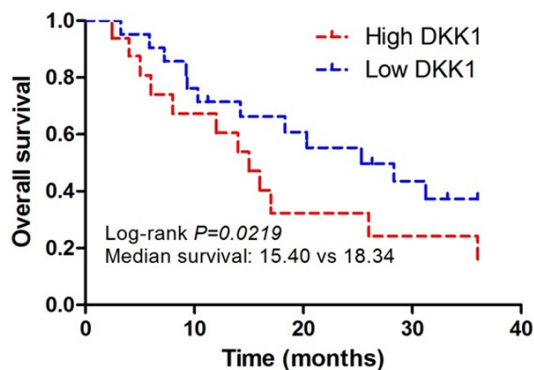


**Figure 5.** Correlation between DKK1 expression and survival time of patients.

when a tumor goes from low malignancy into high malignancy when the tumor size is about 3 cm, it has an increased probability of related gene abnormality, low tumor differentiation and strong invasion, leading to poor prognosis of patients [20]. In this study, the DKK1 protein level in NSCLC patients with a tumor diameter ≥3 cm was higher than those with <3 cm, sug-gesting that the DKK1 protein expression may be related to the changes in tumor diameter in CT imaging of NSCLC patients. The observation of pulmonary nodules by CT can help distin-guish the benign from malignant nodules. It has been proposed that the enhancement degree of 20-60 HU can serve as an index of malignant nodules [21]. It was found in this study that the enhancement value of tumors in the high DKK1 group was remarkably higher than that in group with low levesl of DKK1. The enhancement de-gree of the tumor is associated with the num-ber, thickness and maturity of tumor blood ves-sels [22], indicating that DKK1 may be corre-lated with angiogenesis. In addition, it was found that the DKK1 protein level in tumors of patients with enlargement of hilar and medias-tinal lymph nodes were higher. The possible reason is that cells with high DKK1 expression have potent proliferative activity and strong invasion, and lymph node metastasis occurs easily. However, this study has some limita-tions, based on evidence small sample size with 58 breast cancer patients, so further stud-

ies are warranted using a larger number of samples to validate the findings and ensure the reliability of the results. As previous findings with H1299 and 95C non-small cell lung cancer cell lines have indicated that DKK1 promoted migration and invasion of non-small cell lung cancer via inhibiting the phosphorylation of beta-catenin and resulting in the increased nuclear localization of beta-catenin [23]; as such, there is a continuing need to investigate possible mechanisms of DKK1 in the regulation of NSCLC with further research.

## Conclusion

In conclusion, our data demonstrate that DKK1 is aberrantly overexpressed in serum and tumor tissues of NSCLC patients and is correlated with CT characteristics, which is a predictive index for poor prognosis of patients. The combination of DKK1 detection and CT examination may open new directions for NSCLC diagnosis and treatment.

## Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shujun Cui, Department of Medical Imaging, The First Affiliated Hospital of Hebei North University, No 12, Changqing Road, Qiaoxi District, Zhangjiakou 075000, Hebei, China. Tel: +86-313-8046973; Fax: +86-313-8046973; E-mail: shujuncui102@163.com

## References

[1]     Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK, Veeriah S, Shafi S, Johnson DH, Mitter R, Rosenthal R, Salm M, Horswell S, Escudero M, Matthews N, Rowan A, Chambers T, Moore DA, Turajlic S, Xu H, Lee SM, Forster MD, Ahmad T, Hiley CT, Abbosh C, Falzon M, Borg E, Marafioti T, Lawrence D, Hayward M, Kolvekar S, Panagiotopoulos N, Janes SM, Thakrar R, Ahmed A, Blackhall F, Summers Y, Shah R, Joseph L, Quinn AM, Crosbie PA, Naidu B, Middleton G, Langman G, Trotter S, Nicolson M, Remmen H, Kerr K, Chetty M, Gomersall L, Fennell DA, Nakas A, Rathinam S, Anand G, Khan S, Russell P, Ezhil V, Ismail B, Irvin-Sellers M, Prakash V, Lester JF, Kornaszewska M, Attanoos R, Adams H, Davies H, Dentro S, Taniere P, O'Sullivan B, Lowe HL, Hartley JA, Iles N, Bell H, Ngai Y, Shaw JA, Herrero J, Szallasi Z, Schwarz RF, Stewart A, Quezada SA, Le Quesne J, Van Loo P, Dive C, Hackshaw A and Swanton C; TRACERx Consortium. Tracking the evolution of non-small-cell lung cancer. N Engl J Med 2017; 376: 2109-2121.

[2]     Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L, Komaki R, Lackner RP, Lanuti M, Leal TA, Leisch LJ, Lilenbaum R, Lin J, Loo BW Jr, Martins R, Otterson GA, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Stevenson J, Swanson SJ, Tauer K, Yang SC, Gregory K and Hughes M. Non-small cell lung cancer, version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2017; 15: 504-535.

[3]     Cao W, Yan C, Wang H, Tang T, Wang H and Liu D. Validity of an NGS-based multiple gene panel in identifying actionable mutations for patients with NSCLC in a Chinese hospital. Oncol Lett 2019; 17: 5425-5434.

[4]     Ke L, Xu M, Jiang X and Sun X. Epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase/Oncogene or C-Ros oncogene 1 (ALK/ROS1) fusions inflict non-small cell lung cancer (NSCLC) female patients older than 60 years of Age. Med Sci Monit 2018; 24: 9364-9369.

[5]     Bredenkamp N, Yang J, Clarke J, Stirparo GG, von Meyenn F, Dietmann S, Baker D, Drummond R, Ren Y, Li D, Wu C, Rostovskaya M, Eminli-Meissner S, Smith A and Guo G. Wnt inhibition facilitates rna-mediated reprogramming of human somatic cells to naive pluripotency. Stem Cell Reports 2019; 13: 1083-1098.

[6]     Wang Y, Singhal U, Qiao Y, Kasputis T, Chung JS, Zhao H, Chammaa F, Belardo JA, Roth TM, Zhang H, Zaslavsky AB, Palapattu GS, Pienta KJ, Chinnaiyan AM, Taichman RS, Cackowski FC and Morgan TM. Wnt signaling drives prostate cancer bone metastatic tropism and invasion. Transl Oncol 2020; 13: 100747.

[7]     Henderson J, Pryzborski S, Stratton R and O'Reilly S. Wnt antagonist DKK-1 levels in systemic sclerosis are lower in skin but not blood and is regulated by microRNA33a-3p. Exp Dermatol 2020.

[8]     Neri F, Dettori D, Incarnato D, Krepelova A, Rapelli S, Maldotti M, Parlato C, Paliogiannis P and Oliviero S. TET1 is a tumour suppressor that inhibits colon cancer growth by derepressing inhibitors of the Wnt pathway. Oncogene 2015; 34: 4168-76.

[9]     Rachner TD, Göbel A, Browne A, Hötzel J, Rauner M and Hofbauer LC. P38 regulates the Wnt inhibitor Dickkopf-1 in breast cancer. Biochem Biophys Res Commun 2015; 466: 728-32.

[10]    Li J, Gong W, Li X, Wan R, Mo F, Zhang Z, Huang P, Hu Z, Lai Z, Lu X and Zhao Y. Recent progress

of Wnt pathway inhibitor dickkopf-1 in liver cancer. J Nanosci Nanotechnol 2018; 18: 5192-5206.

[11] Lu C, Liao Z, Cai M and Zhang G. MicroRNA-320a downregulation mediates human liver cancer cell proliferation through the Wnt/β-catenin signaling pathway. Oncol Lett 2017; 13: 573-578.

[12] Redelman-Sidi G, Binyamin A, Gaeta I, Palm W, Thompson CB, Romesser PB, Lowe SW, Bagul M, Doench JG, Root DE and Glickman MS. The canonical Wnt pathway drives macropinocytosis in cancer. Cancer Res 2018; 78: 4658-4670.

[13] Hong SA, Yoo SH, Lee HH, Sun S, Won HS, Kim O and Ko YH. Prognostic value of Dickkopf-1 and ss-catenin expression in advanced gastric cancer. BMC Cancer 2018; 18: 506.

[14] Xu J, Sadahira T, Kinoshita R, Li SA, Huang P, Wada K, Araki M, Ochiai K, Noguchi H, Sakaguchi M, Nasu Y and Watanabe M. Exogenous DKK-3/REIC inhibits Wnt/β-catenin signaling and cell proliferation in human kidney cancer KPK1. Oncol Lett 2017; 14: 5638-5642.

[15] Jumpertz S, Hennes T, Asare Y, Schütz AK and Bernhagen J. CSN5/JAB1 suppresses the Wnt inhibitor DKK1 in colorectal cancer cells. Cell Signal 2017; 34: 38-46.

[16] Caffo M, Esposito E, Barresi V, Caruso G, Cardali SM, Rinaldi M, Mallamace R, Campolo M, Casili G, Conti A, Germanò A, Cuzzocrea S and Minutoli L. Modulation of Dkk-3 and claudin-5 as new therapeutic strategy in the treatment of meningiomas. Oncotarget 2017; 8: 68280.

[17] Li J, Gong W, Li X, Wan R, Mo F, Zhang Z, Huang P, Hu Z, Lai Z, Lu X and Zhao Y. Recent progress of Wnt pathway inhibitor dickkopf-1 in liver cancer. J Nanosci Nanotechnol 2018; 18: 5192-5206.

[18] Cao HZ, Liu XF, Yang WT, Chen Q and Zheng PS. LGR5 promotes cancer stem cell traits and chemoresistance in cervical cancer. Cell Death Dis 2017; 8: e3039.

[19] Henschke CI, Yip R, Smith JP, Wolf AS, Flores RM, Liang M, Salvatore MM, Liu Y, Xu DM, Yankelevitz DF and International Early Lung Cancer Action Program Investigators. CT screening for lung cancer: part-solid nodules in baseline and annual repeat rounds. AJR Am J Roentgenol 2016; 207: 1176-1184.

[20] Artifon EL, Couto D Jr, Sakai P and da Silveira EB. Prospective evaluation of EUS versus CT scan for staging of ampullary cancer. Gastrointest Endosc 2009; 70: 290-296.

[21] Piersiala K, Akst LM, Hillel AT and Best SR. CT lung screening in patients with laryngeal cancer. Sci Rep 2020; 10: 4676.

[22] Bono P, Wasenius VM, Heikkila P, Lundin J, Jackson DG and Joensuu H. High LYVE-1-positive lymphatic vessel numbers are associated with poor outcome in breast cancer. Clin Cancer Res 2004; 10: 7144-7149.

[23] Zhang J, Zhang X, Zhao X, Jiang M, Gu M, Wang Z and Yue W. DKK1 promotes migration and invasion of non-small cell lung cancer via beta-catenin signaling pathway. Tumour Biol 2017; 39: 1010428317703820.