

Original Article

Study of the clinical significance of DKK1 in the diagnosis and therapeutic monitoring of lung cancer

Hongbo Wu, Jiarong Liu, Xiaoqian Mu, Huijuan Wu, Lijuan Chen, Xiufeng Hu, Jie Liu, Lin Zhang, Yanqiu Zhao

Department of Internal Medicine, The Affiliated Tumor Hospital of Zhengzhou University He'nan Cancer Hospital, Zhengzhou 450008, He'nan Province, China

Received September 6, 2015; Accepted November 23, 2015; Epub January 15, 2016; Published January 30, 2016

Abstract: To investigate the clinical value of Dickkopf1 (DKK1) in diagnosis of lung cancer and chemotherapy Monitoring. Methods 127 cases of lung disease patients as research subjects, according to the nature of the disease, divided into the malignant group (n = 67) and benign disease group (n = 60), then choose 60 cases of healthy people as a control group, according to the cancer patients chemosensitivity, divided the malignant group into sensitive group (n = 54) and drug group (n = 13), then detected the target serum DKK1 concentrations using ELSIA double antibody sandwich method and compared. Up Limits of DKK1 of control patients was as a reference value, calculated malignant group, benign disease group and control group DKK1 expression rate; calculated diagnostic sensitivity and specificity of DKK1 of lung cancer by ROC curve area chart. According to the effect of chemotherapy, patients were divided into remission group, stable group and progress group. Results: DKK1 concentration and positive expression rate of malignant group was higher than that in benign group and the control group (P < 0.05); area under the ROC curve showed that, malignant group the different TNM stage, lymph node metastasis, distant metastasis, the serum DKK1 concentrations significant differences (P < 0.05); sensitive group DKK1 concentrations below resistance group (P < 0.05); remission group DKK1 concentration was less than stable group and progress group (P < 0.05). Conclusion: DKK1 in lung cancer patients showed high expression, it may be an important marker of lung cancer screening and diagnosis and chemotherapy evaluation.

Keywords: DKK1, lung cancer, diagnosis, drug resistance

Introduction

The morbidity of lung cancer has been increasing recently. In male tumor patients, lung cancer had already taken the first position in both morbidity and mortality [1]. Since there are usually few symptoms in the early stage of lung cancer, most patients could not feel about it very early, when they were clinically diagnosed, over 75% of the patients have already reached the TNM III-IV stage [2]. However, this problem may be solved by the applications of new techniques such as the use of tumor biomarkers. Previous reports showed that some antigen biomarkers such as neuron specific enolase (NSE), squamous cell carcinoma antigen (SCC) could be used to help diagnosing lung cancer [3]. However, the sensitivities and specialties of these markers were limited. The Yamabuki's team [4] reported that by the use of gene chip screen, they had identified Dickkopf-1 (DKK1) to

be an important target for the early diagnosis and evaluation of lung and esophagus cancer. DKK1 belongs to the Dickkopfs family. The results seemed to be promising. In order to study the effects of DKK1 in diagnosing and evaluating the progress of lung cancer, our team conducted this study. The details were as follows.

Materials and methods

Clinical samples

From August 2010 to December 2013, 67 cases of lung cancer were chosen as the malignant group. In the same period, 60 cases of benign disease were also studied and set as the benign group. Both oral and written informed consents were got from every patient in this study. This study was approved by the Ethics Committee of the Affiliated tumor Hospital of Zhengzhou University.

Table 1. Details of the patients

Malignant group	
Age (mean ± SD, range, years)	57.9 ± 6.9, 33-74
Gender (male, female)	48, 19
TNM stage	
Stage I	9
Stage II	13
Stage III	24
Stage IV	11
Benign group	
Age (mean ± SD, years)	58.1 ± 7.2, 30-75
Gender (male, female)	45, 15
Disease types	
Pneumonia	29
Pleurisy	18
Tuberculosis (tuberculoma)	13
Normal group	
Age (mean ± SD, years)	57.4 ± 6.5, 32-73
Gender (male, female)	48, 12

All patients had been given a definite diagnosis by the combination of laboratory examination, imageological examination and clinical symptoms. The inclusion criteria for choosing the patients in the malignant group were as follows: (1) all patients were diagnosed with lung cancer for the first time and had not undergone any therapies such as chemotherapy or radiotherapy. (2) The patients should not have any contraindications for receiving chemotherapy. (3) The detailed data for the patient should be complete and easy for following up. There were also exclusion criteria for those patients in the study: (1) the patients also have other malignant tumors. (2) The patients have Ankylosing spondylitis (AS), rheumatoid arthritis (RA) or other disease which may affect the serum concentration of DKK1 [5, 6]. (3) The patients have served hepatic and renal dysfunction or chronic respiratory disorders. After selecting and excluding the patients, finally 67 cases were chosen in the malignant group, the detailed clinical data of the patients were listed in **Table 1**.

Collection of the clinical samples

Every Morning, 5 ml fasting venous blood was taken from all the patients. The blood was centrifuged with the speed of 2500 r/min for 20 minutes and then the supernate (the serum) was taken and restored in the temperature

of -80 centigrade before further study. The collected serum was taken out and put in room temperature for 20 minutes before been tested by ELISA.

Detection for the concentration of DKK1

Serum concentrations of DKK1 were detected by ELISA. The human ELISA kits for DKK1 were bought from Langdun biotechnology Co. Ltd, Shanghai, China (Code: 140607). Microplate Reader was bought from BioRad Company, US. (Type: 550). The levels of DKK1 were detected following the instructions of the ELISA kits. For each sample, three duplications were set. The averages of all duplications were used as the final results.

Definition of “chemotherapy sensitivity”

The definition of “chemotherapy sensitivity” in our study was set by reference to the guidelines in The National Comprehensive Cancer Network (NCCN) [4]. Those who had neoplasm recurrence within 6 months after receiving chemotherapy for the first time were identified to be “chemotherapy resistant”. Others which would not have tumor recurrence until 6 months after the first chemotherapy were considered to be chemotherapy sensitive. According to this definition, 54 patients were divided into the “sensitive group”, 13 belonged to the “resistant group”.

Evaluation of the treating effects by chemotherapy

The chemotherapy effects were evaluated by reference to the “Clinical guidelines of the primary pulmonary carcinoma” [5], which classified the treating effects into four classes: complete remission (CR), significantly reduced (SR), stabilization (S) and progress (P) [5]. In our group, the patients were divided into three groups according to the treating effects: the progress disease group (PD), the stable disease group (SD) and the remission group (CR and SR).

Statistical analysis

All analyses were performed using SPSS v 17.0 software (SPSS Inc, USA). Data are shown as the mean ± standard error of the mean. Statistical significance was evaluated by

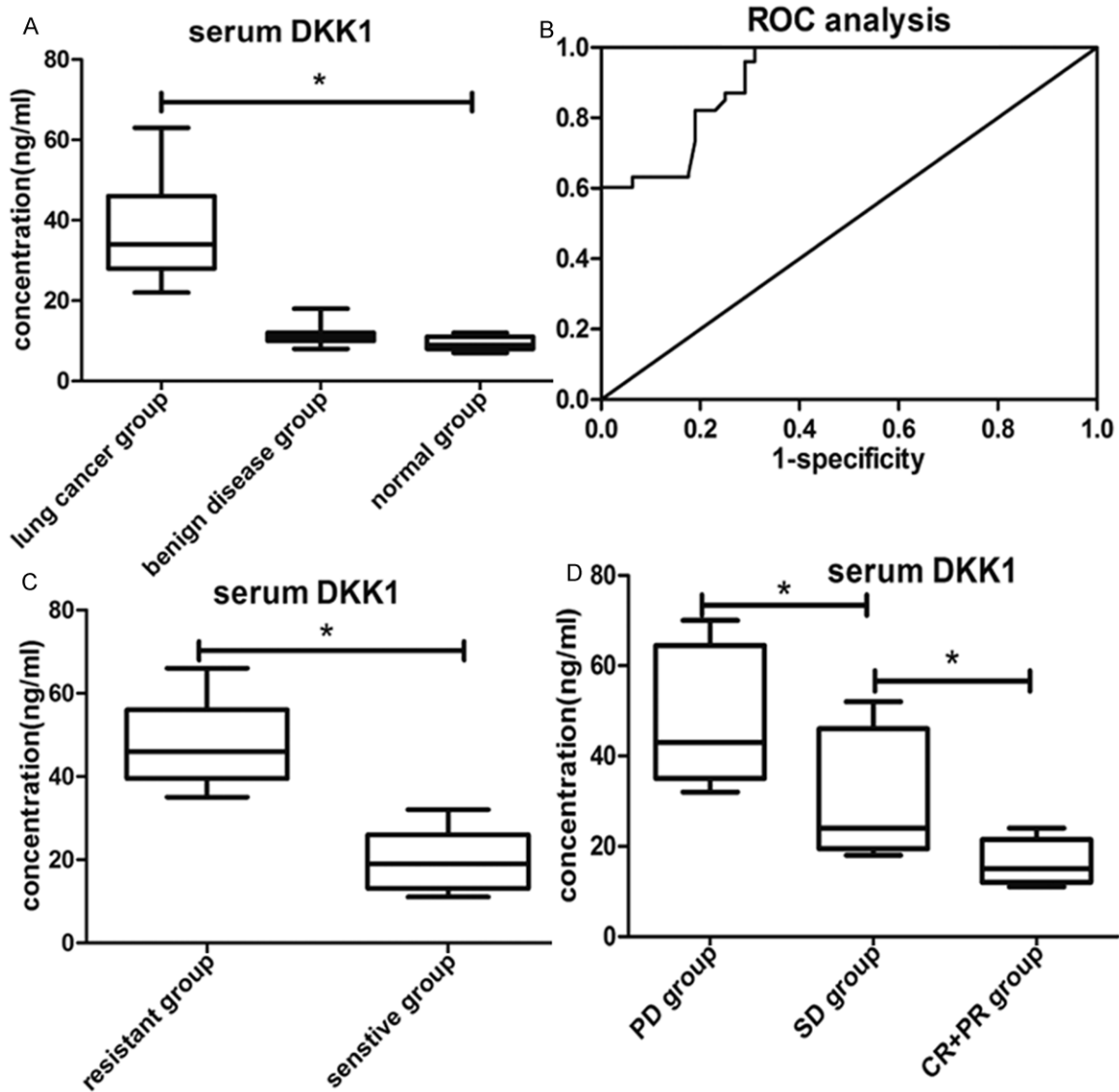


Figure 1. Levels of DKK1 in different groups. A: Serum level of DKK1 in malignant group, benign group and control group. B: ROC curve for the diagnosis of DKK1 for lung cancer. C: Serum DKK1 in sensitive group and resistant group. D: Serum DKK1 in patients with different outcomes.

Table 2. Positive rates of DKK1 in different group

Groups	Cases	Positive cases	Positive rate (%)
Malignant group	67	51	76.12
Benign group	60	4	6.67*
Control group	60	3	5.00*

*: P<0.05 compared with the malignant group.

Student's t test or χ^2 test. P<0.05 was accepted as statistically significant. The sensitivity and specialty were calculated according to the area under ROC curve.

Results

Compartments of the concentrations of DKK1 in different groups

In the malignant group, serum level of DKK1 was 37.45 ± 14.18 ng/mL, significantly higher than those in benign group (10.89 ± 5.41 ng/mL) and control group (9.43 ± 4.27 ng/mL). The differences were statistically significant ($t=13.642$, $P<0.05$). There were no significant difference between the control group and the benign group ($t=1.696$, $P>0.05$). Details were shown in **Figure 1A**.

Table 3. Levels of DKK1 in patients with different clinical background ($\bar{x}\pm s$)

Clinical data	Cases	Level of serum DKK1	t value	P value
gender				
Male	48	38.33 ± 18.65	0.509	0.613
Female	19	35.81 ± 17.23		
Histological type				
Squamous carcinoma	28	36.95 ± 19.14	-0.280	0.781
Adenocarcinoma	39	38.17 ± 16.43		
TNM stages				
Stage I+II	3	27.71 ± 11.53	-4.042	0.000
Stage III+IV	35	43.76 ± 18.58		
Distant metastasis				
Yes	26	50.91 ± 17.06	4.912	0.000
No	41	29.35 ± 17.78		
Lymphatic metastasis				
Yes	31	47.59 ± 17.66	4.737	0.000
No	36	29.25 ± 14.01		

TNM stages, histological types. It was revealed that the concentration of DKK1 was positively connected with TNM stages. Further analysis showed that whether there was metastasis was the key factor. Details were shown in **Table 3**.

Compartment of the levels of DKK1 in sensitive group and resistant group

Serum level of DKK1 in sensitive group was 17.45 ± 6.52 ng/mL and in resistant group it was 41.23 ± 16.79 ng/mL. There were significant differences between the two groups. ($t=-8.266$, $P<0.05$). Details were shown in **Figure 1C**.

The positive rates of DKK1 in different groups

In the control group, the maximum concentration of serum DKK1 was 15.82 ng/mL. 95% of this maximum amount (i.e. 15.03 ng/mL) was set to be the index for evaluating a positive result. Levels ≥ 15.03 ng/mL was defined to be positive and < 15.03 ng/mL was defined to be negative. Based on this setting, in the malignant group, the positive rate was 76.12%, significantly higher than those in the control group (5.00%) and benign group (6.67%). The differences were statistically significant ($\chi^2 = 59.392$, 62.630, $P < 0.05$). The positive rate in the control group and benign group did not have significant differences ($\chi^2 = 0.000$, $P > 0.05$). Details were shown in **Table 2**.

Analysis of the sensitivity and specialty of DKK1 in the diagnosis of lung cancer

According to the results of the area under the ROC curve, the best critical value for the diagnosis of lung cancer was 19.33 ng/mL. The size of the area under the curve was 0.932. The sensitivity of the diagnosis was 83.58% and the specialty was 80.60%. Details were shown in **Figure 1B**.

Compartment of the levels of DKK1 between patients with different TNM stages, histological types

In the malignant groups, the 67 patients were divided into several subgroups according to the

Compartment of the levels of DKK1 in patients with different outcomes after chemotherapy

Serum levels of DKK1 in the remission group was 15.39 ± 4.47 ng/mL, this was significantly lower than those in the stable disease (SD) group (26.54 ± 5.8 ng/mL) and the progress disease (PD) group (46.21 ± 10.78 ng/mL). Details were shown in **Figure 1D**.

Discussion

The WNT-pathway is a very important pathway in regulating the cell signaling transduction. Previous reports showed that this pathway played important roles in the tumorigenesis, differentiation and proliferation [7]. Thus this pathway has been considered to be an important target in the treatment of tumor. In the studies of tumors from the digestive system, activation of WNT pathway contributed to the function of TCF/ β -catenin [8], resulting in up-regulation of the growth of tumor cells and fibroblasts. Except for the effects on cell proliferation, the WNT pathway could also affect cell apoptosis by down-regulating the expression of surviving, which was an important gene in tumorigenesis. Besides, some clinical reports had proved that activation of WNT pathway could contributed to tumor metastasis [9]. Thus, this pathway might directly lead to poor prognosis. Methods targeting this pathway for treating tumor have been constantly studied and discussed in recent years.

A new biomarker for diagnosis

By now, many antagonists against WNT pathway had been studied, including Sfrp, WIF-1, Endostatin and DKK1. Previous reports showed that in healthy people, serum level of DKK1 was quite low [10]. However, Chen [11], Tung [12], et al had showed that in patients with hepatocellular carcinoma, serum levels of DKK1 were significantly higher than those in liver benign patients and normal patients. In Rawson's research [13], it was revealed that the levels of serum DKK1 increased along with the progress of colon cancer. Zhu, et al [14] further tried to use DKK1 in the diagnosis of non-small cell lung cancer and indicated that the sensitivity and specialty of DKK1 were both significantly higher than the commonly used biomarker, CA-125 and CYFRA221-1. This finding showed a promising role for DKK1 in the application of being a good biomarker. Thus, in our study, we made further research and discussions on this.

Our study showed that compared with the control group and benign group, the positive rate of DKK1 was 76.12% in the malignant group, this was significantly higher than those in other groups. Our findings were in accordance with data from previous studies. The sensitivity and specialty for the diagnosis were 83.58% and 80.60%. Both results proved that DKK1 could function as a new marker for the diagnosis of lung malignant tumor.

Except for the diagnosis function, we also further studied the role of DKK1 in evaluating and predicting the progress of lung cancer. Kim's team and Zhang's team [16, 17] had reported that the level of plasma DKK1 had no connections to gender, age but positively related to TNM stages. This finding indicated that DKK1 could be an important role in tumor metastasis. In our team we further compared serum DKK1 in patients with different TNM stages, histological stages in lung cancer and similar results were got. In lung cancer we proved that the level of DKK1 was only positively connected to the TNM stages but no other factors such as age, gender or histological type. Thus it could be concluded that DKK1 could function as a special biomarker in the early warning of lung cancer metastasis.

In clinical diagnosis and treatment, there have been many biomarkers serving as the diagnosis target [17]. On the other hand, the biomark-

ers for predicting the possibilities of chemotherapy-resistance were still very few. The mechanism for the form of chemotherapy-resistance was quite complex and still not very clear. The researchers reported that pharmacokinetics and tumor microenvironment could both be involved in this process [18]. By now some drug-resistance genes had been studied and reported, including Topoisomerase II α , P-glycoprotein, et al [19]. In our study, we found 13 patients who were resistant to chemotherapy. By detecting their concentrations of serum DKK1, we found that they were significantly higher than those who were sensitive to chemotherapies. This finding was in accordance with a previous report [20]. To further study this phenomenon, we also compared the levels of serum DKK1 in patients with different reactions after chemotherapy. It turned out that in the remission group (CR and SR); the levels were significantly lower than those in the progress group (P), the stable group (S). This finding was quite interesting and indicated that DKK1 could not only predict the existence of chemotherapy-resistance but also help indicate the outcomes of the treatment.

In general, our studies showed that serum DKK1 could be a new target in the accurate diagnosis of lung cancer. Also, detecting the level of serum DKK1 could not only help to warn lung cancer metastasis but also predict the treatment outcomes. However, our study was mainly based on clinical outcomes and statistical analysis. The mechanism of the functions of DKK1 still needs to be further discussed. After all, our studies provide a promising role of DKK1 in clinical diagnosis and treatment.

Acknowledgements

This research was funded by the Medical science and technology research projects in Henan Province (200902007 to Yanqiu Zhao); Henan Science and Technology research projects (142102310437 to Hongbo Wu). The authors indicated no potential conflicts of interest.

Disclosure of conflict of interest

None.

Address correspondence to: Yanqiu Zhao, Department of Internal Medicine, The Affiliated Tumor

A new biomarker for diagnosis

Hospital of Zhengzhou University He'nan Cancer Hospital, 127. Dongming Road, Zhengzhou 450008, He'nan Province, China. Tel: 0371-65587657; E-mail: zhaoyanqiu0524@163.com

References

- [1] Kim BG, Kwon HY, Sohn EJ, Hwang S, Kwon OS, Kim SH. Activation of caspases and inhibition of ribosome biogenesis mediate antitumor activity of Chijongdan in A549 non-small lung cancer cells. *BMC Complement Altern Med* 2014; 14: 420.
- [2] Leidinger P, Backes C, Dahmke IN, Galata V, Huwer H, Stehle I, Bals R, Keller A, Meese E. What makes a blood cell based miRNA expression pattern disease specific? - A miRNome analysis of blood cell subsets in lung cancer patients and healthy controls. *Oncotarget* 2014; 5: 9484-97.
- [3] Yamabuki T, Takano A, Hayama S, Ishikawa N, Kato T, Miyamoto M, Ito T, Ito H, Miyagi Y, Nakayama H, Fujita M, Hosokawa M, Tsuchiya E, Kohno N, Kondo S, Nakamura Y, Daigo Y. Dickkopf-1 as a novel serologic and prognostic biomarker for lung and esophageal carcinomas. *Cancer Res* 2007; 67: 2517-25.
- [4] Na Y, Lee SM, Kim DS, Park JY. Promoter methylation of Wnt antagonist DKK1 gene and prognostic value in Korean patients with non-small cell lung cancers. *Cancer Biomark* 2012; 12: 73-9.
- [5] Choi BY, Chang SH, Cho HJ, Kang EH, Shin K, Song YW, Lee YJ. The association of radiographic progression with serum R-spondin 1 (RSPO1) levels or Dickkopf-1 (DKK1)/RSPO1 ratios in rheumatoid arthritis patients: clinical evidence for reciprocal inhibition between DKK1 and RSPO1. *Scand J Rheumatol* 2014; 2: 1-9.
- [6] Guo CC, Zhang XL, Yang B1, Geng J, Peng B, Zheng JH. Decreased expression of Dkk1 and Dkk3 in human clear cell renal cell carcinoma. *Mol Med Rep* 2014; 9: 2367-73.
- [7] Cho SW, Lee EJ, Kim H, Kim SH, Ahn HY, Kim YA, Yi KH, Park do J, Shin CS, Ahn SH, Cho BY, Park YJ. Dickkopf-1 inhibits thyroid cancer cell survival and migration through regulation of β -catenin/E-cadherin signaling. *MolCell Endocrinol* 2013; 366: 90-8.
- [8] Huang Z, Li S, Song W, Li X, Li Q, Zhang Z, Han Y, Zhang X, Miao S, Du R, Wang L. Lysine-specific demethylase 1 (LSD1/KDM1A) contributes to colorectal tumorigenesis via activation of the Wnt/ β -catenin pathway by down-regulating Dickkopf-1 (DKK1) [corrected]. *PLoS One* 2013; 8: e70077.
- [9] Prieto PA, Cha CH. DKK1 as a serum biomarker for hepatocellular carcinoma. *Hepatobiliary SurgNutr* 2013; 2: 127-8.
- [10] Pozzi S, Fulciniti M, Yan H, Vallet S, Eda H, Patel K, Santo L, Cirstea D, Hideshima T, Schirtzinger L, Kuhstoss S, Anderson KC, Munshi N, Scadden D, Kronenberg HM, Raju N. In vivo and in vitro effects of a novel anti-Dkk1 neutralizing antibody in multiple myeloma. *Bone* 2013; 53: 487-96.
- [11] Chen L, Li M, Li Q, Wang CJ, Xie SQ. DKK1 promotes hepatocellular carcinoma cell migration and invasion through β -catenin/MMP7 signaling pathway. *Mol Cancer* 2013; 12: 157.
- [12] Tung EK, Ng IO. Significance of serum DKK1 as a diagnostic biomarker in hepatocellular carcinoma. *Future Oncol* 2012; 8: 1525-8.
- [13] Cedrés S, Nuñez I, Longo M, Martínez P, Checa E, Torrejón D, Felip E. Serum tumor markers CEA, CYFRA21-1, and CA-125 are associated with worse prognosis in advanced non-small-cell lung cancer (NSCLC). *Clin Lung Cancer* 2011; 12: 172-9.
- [14] Krause U, Ryan DM, Clough BH, Gregory CA. An unexpected role for a Wnt-inhibitor: Dickkopf-1 triggers a novel cancer survival mechanism through modulation of aldehyde-dehydrogenase-1 activity. *Cell Death Dis* 2014; 5: e1093.
- [15] Chu T, Teng J, Jiang L, Zhong H, Han B. Lung cancer-derived Dickkopf1 is associated with bone metastasis and the mechanism involves the inhibition of osteoblast differentiation. *BiochemBiophys Res Commun* 2014; 443: 962-8.
- [16] Kim IG, Kim SY, Kim HA, Kim JY, Lee JH, Choi SI, Han JR, Kim KC, Cho EW. Disturbance of DKK1 level is partly involved in survival of lung cancer cells via regulation of ROMO1 and γ -radiation sensitivity. *BiochemBiophys Res Commun* 2014; 443: 49-55.
- [17] Zhang H, Yu C, Dai J, Keller JM, Hua A, Sottnik JL, Shelley G, Hall CL, Park SI, Yao Z, Zhang J, McCauley LK, Keller ET. Parathyroid hormone-related protein inhibits DKK1 expression through c-Jun-mediated inhibition of β -catenin activation of the DKK1 promoter in prostate cancer. *Oncogene* 2014; 33: 2464-77.
- [18] Papadoniou N, Kosmas C, Gennatas K, Polyzos A, Mouratidou D, Skopelitis E, Tzivras M, Sougioultzis S, Papastratis G, Karatzas G, Papalambros E, Tsavaris N. Prognostic factors in patients with locally advanced (unresectable) or metastatic pancreatic adenocarcinoma: a retrospective analysis. *Anticancer Res* 2008; 28: 543-9.
- [19] Liu Y, Tang W, Xie L, Wang J, Deng Y, Peng Q, Zhai L, Li S, Qin X. Prognostic significance of dickkopf-1 overexpression in solid tumors: a

A new biomarker for diagnosis

- meta-analysis. *Tumour Biol* 2014; 35: 3145-54.
- [20] Rawson JB, Sun Z, Dicks E, Daftary D, Parfrey PS, Green RC, Gallinger S, McLaughlin JR, Wang PP, Knight JA, Bapat B. Vitamin D Intake Is Negatively Associated with Promoter Methylation of the Wnt Antagonist Gene DKK1 in a Large Group of Colorectal Cancer Patients. *Nutr Cancer* 2012; 64: 919-28.