RESEARCH ARTICLE

GOLPH3, a Good Prognostic Indicator in Early-stage NSCLC Related to Tumor Angiogenesis

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Abstract

Background: Golgi phosphoprotein-3 (GOLPH3) is implicated in cancer development and progression. The aim of this study was to evaluate the prognostic significance of GOLPH3 protein and its association with tumor angiogenesis in patients with early-stage NSCLC. Materials and Methods: Immunohistochemistry was performed to determine GOLPH3 protein expression and allow assessment of intratumoral microvessel density (MVD) by counting CD-34 positive immunostained endothelial cells. Correlations of expression with MVD, clinicopathologic features and clinical prognosis were analyzed. Results: A notably higher level of GOLPH3 expression was found in early-stage NSCC tissues at the protein level. However, we do not find any correlation between GOLPH3 expression and clinicopathologic features ($p>0.05$), although higher MVD was positively associated with GOLPH3 overexpression ($p<0.001$). Expression of GOLPH3 was found to be an independent prognostic factor in early-stage NSCLC patients, those expressing high levels of GOLPH3 exhibiting a substantially lower 5-year overall survival than GOLPH3-negative patients (adjusted HR =1.899, 95% CI: 1.021-3.532, $p=0.043$). Conclusions: High expression of the GOLPH3 protein is common in early-stage NSCC, and is closely associated with tumor progression, increased tumor angiogenesis, and poor survival. We conclude a possibility of its use as a diagnostic and prognostic marker in early-stage NSCC patients.

Keywords: GOLPH3 - non-small cell lung cancer - angiogenesis - prognosis

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Introduction

Lung cancer is the leading cause of death among the malignant tumors worldwide, and the incidence of lung cancer is increasing (Travis, 2011). Non-small-cell lung cancer (NSCLC) accounts for up to 80 % of all lung cancer cases (Brognard et al., 2001; Ren et al., 2004). Despite great advance in the treatment of cancers in recent years, the prognosis for patients with lung cancer remains poor, with 5-year survival rates less than 15% (Smith et al., 2010; Gao et al., 2011). Recurrence and the emergence of metastases are major causes of therapeutic failure in cancer patients, and 30-40% of stage I patients relapse after surgical resection (Fry et al., 1999). Moreover, prospective randomized data showed that adjuvant chemotherapy in stage IB has not achieved a significant survival benefit and even a detrimental effect was observed in stage IA (Crino et al., 1999). However, the selection criteria for use of adjuvant chemotherapy in patients with stage I NSCLC have not been established, only 4% of patients may actually benefit from such adjuvant therapy, while most patients will suffer undesired toxicity or potentially fatal side-effects, without gaining benefit in survival and quality of life (Arriagada et al., 2004; Winton et al., 2005; Pignon et al., 2006). Therefore, there is an urgent need to identify novel biomarkers that will help select the patients with high chance of lung cancer recurrence and provide better prognosis and individualization treatment.

Golgi phosphoprotein-3 (GOLPH3), also known as GPP34/GMx33/MIDAS, is a highly conserved protein with molecular weight of 34 kDa, which plays a role in anterograde and retrograde Golgi trafficking (Wu et al., 2000; Nakashima et al., 2005; Snyder et al., 2006). GOLPH3 localizes in human chromosome 5p13, a region that is requently amplified in multiple solid tumor types (Bohm et al., 2002; Yokoi et al., 2002; Gorringe et al., 2005). Simultaneously, GOLPH3 is highly expressed in several solid tumors, including melanoma, colon adenocarcinoma, breast cancer and non-small cell lung cancer (Scott et al., 2009; Zeng et al., 2012). Recently, GOLPH3 has been shown to be involved in tumorigenesis by activating mTOR signaling, enhancing AKT activity, as well as decreasing FOXO1 transcriptional activity (Zeng et al., 2012). Moreover, a series of studies indicated that GOLPH3 overexpression correlates with poor prognosis in patients with breast cancer, esophageal squamous cell

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carcinoma (ESCC), gastric cancer, oral tongue cancer, prostate cancer, and glioblastoma multiforme (Zeng et al., 2012; Wang et al., 2012; Li et al., 2012; Hua et al., 2012; Zhou et al., 2012; Wang et al., 2013; Hu BS et al., 2013). In addition, the results indicate that GOLPH3 might be an effective molecular target for gene therapy in esophageal squamous cancer (Wang et al., 2013).

However, research studies have seldom studied the correlation between GOLPH3 expression and prognosis of Chinese patients with non-small cell lung cancer, specifically, the early-stage non-small cell lung cancer. Angiogenesis is an early event in carcinogenesis, and it plays an essential role in the processes of invasion and metastasis. Studies have shown that high MVD by CD34 is more closely related to poor survival than the other neoangiogenetic factors in stage IB-IIA NSCLC, because that these factors are more closely related to the metastatic process (Yano et al., 2000; Mineo et al., 2004). The correlation between GOLPH3 expression and prognosis suggested that GOLPH3 contribute to NSCLC invasion and metastasis by contributing to increased angiogenesis. To the best of our knowledge, this is the firstly made the study of relationship between GOLPH3 and cancer angiogenesis, and we believe that we have made some valuable progress.

Thus, to determine the role of GOLPH3 protein in the biologic behavior of early-stage NSCLC, we employed immunohistochemical method to examine GOLPH3 protein expression in clinical NSCLC samples, and we analyzed the relationships of GOLPH3 expression with variable clinicopathologic features and patient prognosis. Moreover, we investigated the possible angiogenic role by comparing GOLPH3 protein expression with intratumoral microvessel density (MVD) and assessed the independent prognostic factors affecting long-term survival.

Materials and Methods

Materials

A total of 116 consecutive patients who were diagnosed with Stage I NSCLC and treated with pulmonary lobectomy plus regional lymph node dissection from January 2000 through December 2001 at the Department of Thoracic Surgery, Qilu Hospital, were studied retrospectively. And they all had the clear pathological diagnosis without preoperatively radiotherapy and chemotherapy, and had no distant metastases. The data on their clinicopathologic features and follow-up were complete. And 57 cases were taken more than 5 cm from the tumor margin of normal lung tissues as a negative control.

The clinical characteristics of the patient are summarized in Table 1. For all patients, histological type and grade of cancer cell differentiation were reevaluated and determined by the classification system of the World Health Organization modified in 2004, and postsurgical pathological staging was determined based on the international staging system. Clinical follow-up data was available for a minimum of 5 years or until death. Informed consent was obtained from all patients and this study was approved by the Ethics Committee of Qilu Hospital (Shandong, China).

Immunohistochemistry

All specimens were collected during the surgery, fixed by 10% formalin and embedded in paraffin. The tissues were cut as 4 μm serial sections, and then deparaffinized using xylene and rehydrated through an ethanol series to water. High-temperature antigen retrieval was carried out in citrate buffer for 25 min in a microwave oven. Then the endogenous peroxidase enzyme activity was blocked using 3% H2O2 in methanol for 25 min at room temperature. The slides were then incubated with primary rabbit anti-GOLPH3 polyclonal antibody (Abcam) and rabbit anti-CD34 monoclonal antibody (Santa Cruz Biotechnology) overnight at 4°C in a high humidity chamber, followed by incubation for 45 min at 37°C with biotinylated secondary antibodies and streptavidin-peroxidase complex. Finally, a 3, 30-diaminobenzidine solution was added, and the slides were counterstained with hematoxylin and mounted with neutral balsam. For negative controls, sections were incubated with PBS instead of the primary antibodies.

Evaluation of immunohistochemical staning

All sections were reviewed independently by three independent observers blinded to all clinical and pathologic information. Discordant cases were resolved by choosing the value consistent between two observers or the average of the scores. A reproducible semi-quantitative method that considered both staining intensity (0, negative; 1, weak; 2, moderate and 3, strong) and the percentage of positively stained cells (0, 0-5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; 4, >76%) was adopted (Vermeulen PB et al., 2002) The staining index (SI) was calculated by multiplying the product of the staining intensity score and the proportion of positive tumor cells. For intratumoral microvessel density (MVD), microvessels were recorded by counting CD34 positively stained endothelial cells as descripted previously. The microvessel count (MVC) was determined independently by two pathologists in each case. Three representative areas of dense neovascularization were selected under low microscope power (×10 objective lens and ×10 ocular lens) and then vessels were counted at higher magnification (×20 objective lens and ×20 ocular lens). The average of the counts in three fields was recorded. Large vessels with thick, muscular walls were excluded, implementation of the calculation results are rounded.

The cutoff value for high and low expression was determined based on a heterogeneity value measured through log-rank statistical analysis with respect to overall survival (Dominik et al., 2005).

Statistical Analysis

The association between GOLPH3 expression and clinicopathological variables was plotted by the chi-square test. The correlation between intratumoral MVD and a patient's clinicopathologic factors and GOLPH3 protein immunoreactivity was analyzed by nonparametric test (Mann-Whitney U test or Kruskal-Wallis H test). Kaplan-Meier method was used to calculate the survival curves, and log-rank test was used to compare the difference between the survivals of patient subgroups. Multivariate Cox regression analysis was used to identify significant

Table 1. Correlations of Clinicopathologic Variables of Early-stage NSCLC with GOLPH3 and MVD

Variable		GOLPH3 (overexpression)				MVD	
		No. of patients	yes	no	P^a	Median (interquartile range)	P^b
Age	≤65 years	58	29	29	0.059	49.50 (33.00-59.75)	0.596
	>65 years	58	39	19		48.50 (35.00-62.00)	
Gender	Male	68	42	26	0.413	49.00 (34.00-60.50)	0.991
	Female	48	26	22		48.50 (34.25-63.50)	
Differentiation	Well	31	17	14	0.852	39.00 (32.00-62.00)	0.465
	Moderate	44	27	17		51.50 (35.00-58.00)	
	Poor	41	24	17		48.00 (35.50-65.50)	
T classification	T1	46	24	22	0.253	53.00 (36.75-63.00)	0.031
	T2	70	44	26		41.00 (33.00-56.00)	
Histology	Adeno	66	35	31	0.16	48.00 (35.75-62.00)	0.486
	Squamous	50	33	17		49.50 (32.00-61.25)	

*P^a Chi-square test; P^b Nonparametric test (Mann- Whitney U test or Kruskal-Wallis H test)

Table 2. Expression of GOLPH3 in Lung Cancer, Adjacent Lung Cancer Tissues and Normal Tissues

Variable	n	Positive (n)	Positive rate (%)	χ^2	*P*
Cancer tissue	116	68	58.6%	4.187	0.041
Normal tissue	57	24	42.1%		

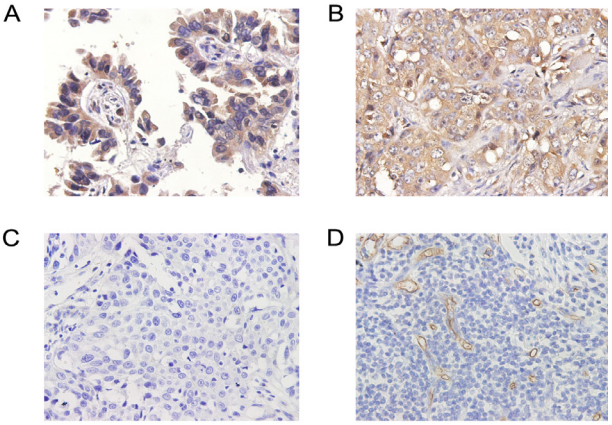

Figure 1. Expression Pattern of GOLPH3 in lung cancer tissues. (a) High GOLPH3 expression in lung adenocarcinoma. (b): High GOLPH3 expression in lung squamous cell carcinoma tissues. (c): Negative GOLPH3 expression in NSCLC tissues. (d): Intratumoral microvessels were stained as brown by the anti-CD34 monoclonal antibody in lung cancer tissues (magnification×400).

independent prognostic factors. Differences between groups were considered significant for *P* value<0.05. All statistical analyses were performed with SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Expression of GOLPH3 in Early-Stage NSCLC and its relation to clinicopathologic profiles

Immunohistochemical staining for GOLPH3 was performed in 116 tumors and 57 normal lung tissues. As shown in Figure. 1 A and B, immunohistochemical analysis showed that the GOLPH3 protein was immunostained in the cancer cell cytoplasm. The correlation of GOLPH3 overexpression with clinicopathologic features was analyzed. Table 1 summarized the association of GOLPH3 expression with various clinicopathological features of early-stage NSCLC tissues. However, we observed no

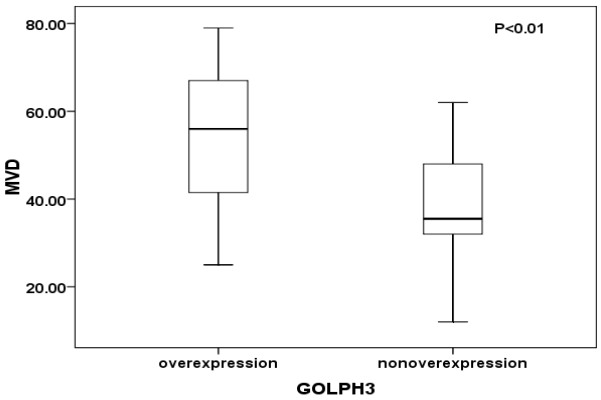

Figure 2. Intratumoral Microvessel Density in Relation to GOLPH3 Protein Immunoreactivity. Mann-WhitneyUtest demonstrated that tumors with GOLPH3 protein high expression showed significantly higher intratumoral MVD than tumors with GOLPH3 protein low expression (*P*< 0.01)

statistically significant correlation between GOLPH3 protein expression and any clinicopathological features of NSCLC tissues (*p*> 0.05, Table 1). The mean value of GOLPH3 overexpression in 116 early-stage NSCLC tissues was 58.62 %, significantly higher than normal lung tissues (42.11%, *p*= 0.041; Table 2).

Correlation between MVD and clinicopathologic factors of Early-Stage NSCLC

Intratumoral MVD was quantified by counting CD34-positive endothelial cells in cancer tissues (Figure. 1D), and the staining intensity of MVD ranged broadly from 12 to 79 microvessels/ 200×magnification field (Median: 47.69/HPF). Meanwhile, we found that MVD was significantly correlated with invasion depth (*p*=0.031, Mann-Whitney U test), while no significant correlations were observed between MVD and other clinicopathologic factors (*p*>0.05, Table 1).

Correlation of GOLPH3 Protein with MVD

Statistical analysis demonstrated that there was significantly more MVD in tumors with GOLPH3 protein high expression than that in those with GOLPH3 protein low expression (*p*<0.001, Mann-Whitney U test; Figure. 2).

Univariate and multivariate survival analysis

In order to investigate the prognostic implications of

Table 3. Univariate and Multivariate Analyses of Prognostic Variables

	PFS Univariate analysis	PFS Multivariate analysis		
Variable	P value (log-rank test)	95.0% confidence interval	Exp (B)	*P* value
Gende	0.304	0.461 -1.250	0.759	0.279
Age	0.31	0.727 -1.928	1.184	0.497
T classification	0.183	0.906 -2.644	1.548	0.11
Differentiation	0.474	0.777 -1.452	1.062	0.707
Histology	0.484	0.641 -1.711	1.047	0.854
GOLPH3 protein	0.001	1.034 -3.251	1.833	0.038
MVD	0.014	1.017 -3.127	1.783	0.044

	OS Univariate analysis	OS Multivariate analysis		
Variable	P value (log-rank test)	95.0% confidence interval	Exp (B)	*P* value
Gender	0.273	0.440 -1.270	0.748	0.282
Age	0.352	0.709 -2.005	1.192	0.508
T classification	0.115	0.947 -2.996	1.684	0.076
Differentiation	0.343	0.647 -1.267	0.905	0.562
Histology	0.26	0.689 -1.960	1.162	0.575
GOLPH3 protein	0.001	1.021 -3.532	1.899	0.043
MVD	0.019	0.994 -3.284	1.806	0.053

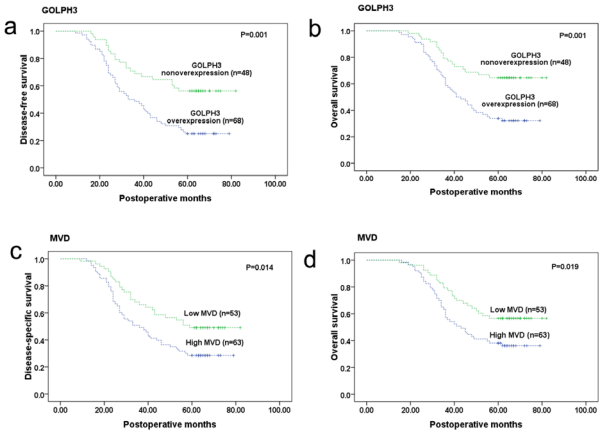

Figure 3. Kaplan-Meier Curves of Disease-Free and Overall Survival Stratified According to the Status of GOLPH3 Protein Expression and MVD

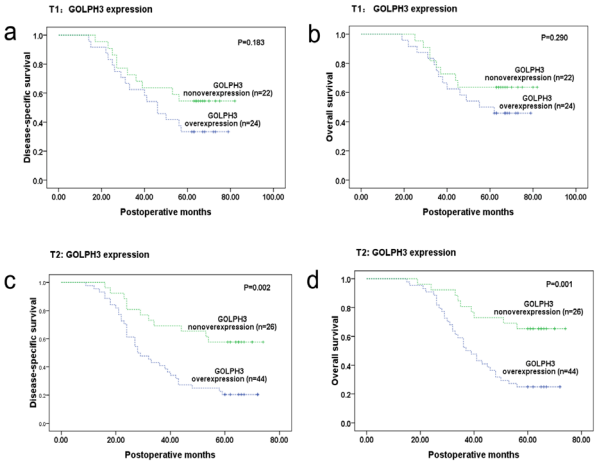

Figure 4. Kaplan-Meier Survival Curves of Patients Stratified According to Invasion Depth.

GOLPH3 expression in overall survival and progression-free survival of early-stage NSCLC, the detailed clinical information of all 116 NSCLC patients in high GOLPH3 expression and low GOLPH32 expression groups was reviewed. At the time of this analysis, with a median follows up of 52.00 months (range 15-82 months), 53 patients (45.69%) were alive and 44 patients (37.93%) remained free of disease. As determined by the log-rank test, patients with positive GOLPH3 expression showed a more unfavorable prognosis than those with no over expression (p=0.001, Figure 3A). Meanwhile, the patients with high GOLPH3 expression have a high rate of tumor recurrence (p=0.001, Figure 3B). Moreover, the univariate analysis revealed that the high MVD predicted poorer overall and progression-free survival of NSCLC patients (p =0.019 and p = 0.014, resp., Table 3, Figure 3C and D). Furthermore, the multivariate analyses identified GOLPH3 protein overexpression and high MVD as independent prognostic factors for progression-free survival (p= 0.038 and p= 0.044, resp., Table 3). However, only the GOLPH3 protein overexpression retained its significance as an independent prognosticator for distasteful overall as well as progression-free survival (p= 0.043, Table 3).

We further analyzed the prognostic significance of GOLPH3 protein in selective patient subgroups stratified according to the invasion depth of NSCLC. Univariate analysis demonstrated that the overall 5-year survival and progression-free 5-year survival rate of patients with GOLPH3 protein high expression was significantly lower than that of the remaining patients among T2 (p= 0.001 and P=0.002, respectively; Figure. 4). However, this trend was not found in patients with T1 invasion depth (p=0.290 and p=0.183, respectively; Figure.4).

Discussion

The mammalian target of rapamycin (mTOR) has emerged as a critical effector in cell-signaling pathways commonly deregulated in human cancers. This has led to the prediction that mTOR inhibitors may be useful in oncology, and derivatives of one such molecule, rapamycin (from which mTOR derives its name), are currently in clinical development. And it has been reported that overexpression of GOLPH3 promotes cell transformation

via enhancing the activity of the serine/threonine kinase mTOR. Recent studies have found that the development of a variety of solid tumors abnormally activation is related with mTOR signal transduction pathways in breast cancer, glioma and so on, so the GOLPH3 has become an important target for cancer therapy (Guertin et al., 2007; Scott et al., 2009). However, few studies have investigated the expression and significance of GOLPH3 in early-stage NSCC, especially for the prognosis of early-stage NSCLC. In the present study, we focused on early-stage NSCLC cancer patients to determine the correlation of GOLPH3 expression with clinicopathological parameters and survival.

Furthermore, we found that high GOLPH3 expression and high MVD were strongly associated with decreased overall survival and progression-free survival in early-stage NSCLC patients. In multivariable analysis, GOLPH3 expression and MVD retained its independence factors in predicting progression-free survival for NSCLC patients, suggesting that GOLPH3 protein and MVD may be potential prognostic factors for the relapse of early-stage NSCLC patients. However, in multivariate analysis, only GOLPH3 expression could independently and significantly predict overall 5-year survival. These findings provide evidence that GOLPH3 could be regarded as a biomarker for predicting the outcome of early-stage NSCLC patients.

In this study, our results showed that there was no significant correlation between GOLPH3 expression and the clinicopathological features of early-stage NSCLC. However, high MVD was significantly associated with T status in early-stage NSCLC, but not associated with other clinicopathological features. To a certain extent, T stage is a critical process of tumor development, and the difference in the correlation between MVD and clinicopathological features may reflect that the microvessel density is an important aspect of tumor development. And angiogenesis plays a vital role in tumor initiation, progression and metastasis, and it has been considered one of the hallmarks of cancer (Butler et al., 2010; Hanahan et al., 2011). We studied the relationship between the expression of GOLPH and MVD. Notably, our results showed that the overexpression of GOLPH3 significantly associated with increased angiogenic activity measured as intratumoral MVD, suggesting that GOLPH3 plays crucial role in NSCLC tumorigenesis by the induction or/and promotion of tumor angiogenesis. Furthermore, the patients with positive GOLPH3 expression showed a more unfavorable prognosis than those with no over-expression in T2 invasion depth of NSCLC, not in T1 stage. These findings suggest that the carcinogenicity of GOLPH3 protein may be an independent carcinogenic factor, working along with the tumor progression gradually. Although there is no definitive finding on the regulatory mechanism of angiogenesis by GOLPH3, our results highlight the potential role of GOLPH3 in tumor angiogenesis.

Taken together, our data support the assumption that GOLPH3 protein over-expression is common in early-stage NSCLC and significantly correlated with tumor angiogenesis. Moreover, it is an independent prognostic factor for early-stage NSCLC patients.

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References

Arriagada R, Bergman B, Dunant A, et al (2004). Cisplatin-based adjuvant chemotherapy in patients with completely resected non- small-cell lung cancer. *N Engl J Med*, **350**, 351-60.

Brognard J, Clark AS, Ni Y et al (2001). Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. *Cancer Res*, **61**, 3986-97.

Bohm M, Wieland I, Schmidt C, et al (2001). Loss of heterozygosity on chromosome 5p13-12 predicts adverse prognosis in advanced bladder cancer independent of tumor stage and grade. *J Urol* , **168**, 2655-58.

Butler JM, Kobayashi H, Rafii S (2010). Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. *Nat Rev Cancer*, **10**, 138-46.

Crino` L, Weder W, van Meerbeeck J, et al (2001). Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, **21**, 103-15.

Dominik Wolf, Anna M.Wolf, Holger Rumpold, et al (2005). The expression of the regulatory T cell-specific forkhead box transcription factor Foxp3 is associated with poor prognosis in ovarian cancer. *Clin Cancer Res*, **11**, 8326-31.

Fry WA, Philips JL and Menck HR (1999). Ten-year survey of lung cancer treatment and survival in hospitals in the United States: a national cancer data base report. *Cancer*, **86**, 1867-76.

Gao W, Liu L, Lu X, et al (2011). Circulating microRNAs: possible prediction biomarkers for personalized therapy of non-small-cell lung carcinoma. *Clin Lung Cancer*, **12**, 14-7.

Gorringe KL, Boussioutas A, Bowtell DD (2005). Novel regions of chromosomal amplification at 6p21, 5p13, and 12q14 in gastric cancer identified by array comparative genomic hybridization. *Genes Chromosomes Cancer*, **42**, 247-59.

Guertin D A, Sabatini D M (2007). Defining the role of mTOR in cancer. *Cancer cell*, **12**, 9-22.

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell*, **144**, 646-74.

Hua X, Yu L, Pan W, et al (2012). Increased expression of Golgi phosphoprotein-3 is associated with tumor aggressiveness and poor prognosis of prostate cancer. *Diagn Pathol* , **7**, 127.

Hu BS, Hu H, Zhu CY, et al (2013). Overexpression of GOLPH3 is associated with poor clinical outcome in gastric cancer. *Tumour Biol*, **34**, 515-20.

Li H, Guo L, Chen SW, et al (2012). GOLPH3 overexpression correlates with tumor progression and poor prognosis in patients with clinically N0 oral tongue cancer. *J Transl Med*, **10**, 168.

Mineo T C, Ambrogi V, Baldi A, et al (2004). Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. *J Clin Pathol*, **57**, 591-97.

Nakashima KN, Asoh S, Ishibashi Y, et al (2005). MIDAS/GPP34, a nuclear gene product, regulates total mitochondrial mass in response to mitochondrial dysfunction. *J Cell Sci*, **118**, 5357-67.

Pignon JP, Tribodet H, Scagliotti GV, et al, on behalf of the LACE Collaborative Group (2006). Lung Adjuvant Cisplatin Evaluation (LACE): a pooled analysis of five randomized clinical trials including 4, 584 patients. *J Clin Oncol*, 24, 366s (abstract 7008).

Ren H, Tang X, Lee JJ, et al (2004). Expression of hepatoma-derived growth factor is a strong prognostic predictor for patients with early-stage non-small-cell lung cancer. *J Clin Oncol*, **22**, 3230-37.

Snyder CM, Mardones GA, Ladinsky MS, et al (2006). GMx33 associates with the trans-Golgi matrix in a dynamic manner and sorts within tubules exiting the Golgi. *Mol Biol Cell,* **17**, 511-24.

Smith CB, Kelley AS, Meier DE (2010). Evidence for new standard of care in non-small cell lung cancer patients. *Semin Thorac Cardiovasc Surg* , **22**, 193-4.

Scott KL, Kabbarah O, Liang MC, et al (2009). GOLPH3 modulates mTOR signalling and rapamycin sensitivity in cancer. *Nature*, **459**, 1085-90.

TravisWD (2011). Pathology of lung cancer. *Clin Chest Med*, **32**, 669-92.

Vermeulen PB, Gasparini G, Fox SB, et al (2002). Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur J Cancer*, **38**, 1564-79.

Wu CC, Taylor RS, Lane DR, et al (2000). GMx33: a novel family of trans-Golgi proteins identified by proteomics. *Traffic*, **1**, 963-75.

Winton T, Livingston R, Johnson D, et al (2005). Vinorelbine plus cisplatin *vs* observation in resected non-small-cell lung cancer. *N Engl J Med*, **352**, 2589-97.

Wang JH, Chen XT, Wen ZS, et al (2012). High expression of GOLPH3 in esophageal squamous cell carcinoma correlates with poor prognosis. *PLoS One*, **7**, 45622.

Wang Q, Wang X, Zhang C B (2013). Lentivirus mediated GOLPH3 shRNA inhibits growth and metastasis of esophageal squamous cancer. *Asian Pac J Cancer Prev*, **14**, 5391-96.

Yokoi S, Yasui K, Saito-Ohara F, et al (2002). A novel target gene, SKP2, within the 5p13 amplicon that is frequently detected in small cell lung cancers. *Am J Pathol*, **161**, 207-16.

Yano T, Tanikawa S, Fujie T, et al (2002). Vascular endothelial growth factor expression and neovascularisation in non-small cell lung cancer. *Eur J Cancer*, **36**, 601-9.

Zeng Z, Lin H, Zhao X, et al (2012). Overexpression of GOLPH3 promotes proliferation and tumorigenicity in breast cancer via suppression of the FOXO1 transcription factor. *Clin Cancer Res*, **18**, 4059-69.

Zhou J, Xu T, Qin R, et al (2012). Overexpression of Golgi phosphoprotein-3 (GOLPH3) in glioblastoma multiforme is associated with worse prognosis. *J Neurooncol*, **110**, 195-203.