Original Paper

Prognostic Significance of LncRNA PVT1 and Its Potential Target Gene Network in Human Cancers: a Comprehensive Inquiry Based Upon 21 Cancer Types and 9972 Cases

Rong-quan He^a Mei-jiao Qin^a Peng Lin^b Yi-Huan Luo^c Jie Ma^a Hong Yang^b
Xiao-hua Hu^a Gang Chen^d

^aDepartment of Medical Oncology, the First Affiliated Hospital of Guangxi Medical University, Nanning,

^bDepartment of Medical Ultrasonography, the First Affiliated Hospital of Guangxi Medical University,

Nanning, ^cDepartment of Gastrointestinal Surgery, the First Affiliated Hospital of Guangxi Medical

University, Nanning, ^dDepartment of Pathology, the First Affiliated Hospital of Guangxi Medical University, Nanning, China

Key Words

Pvt1 • Cancer • Prognosis • Pathway • LncRNA

Abstract

Background/Aims: Whether the level of long noncoding RNA plasmacytoma variant translocation 1 gene (lncRNA PVT1) expression influences the clinical development and outcome of human cancers has not been thoroughly elucidated to date. Inconsistencies still exist regarding the associations between PVT1 and the clinicopathological features, including patient survival data. Additionally, the regulatory mechanism of PVT1 among human cancers remains unclear. **Methods:** we conducted a comprehensive inquiry to verify the implication of PVT1 expression in cancer patients by conducting a meta-analysis of 19 selected studies and The Cancer Genome Atlas (TCGA) database to examine the relationship between PVT1 expression and both the prognosis and clinicopathological features of cancer patients using STATA 12.0. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the potential mRNA target genes of PVT1 gathered from TANRIC and Multi Experiment Matrix (MEM) were performed. **Results:** The level of PVT1 expression in tumor tissues was higher than in paired non-cancer tissues and was significantly associated with a poorer prognosis in cancer patients. Additionally, overexpression of PVT1 was significantly correlated with histological differentiation, tumor (T) classification, lymph node (N) classification and TNM stages. Furthermore, a total of 462 validated target genes were identified, and the GO and KEGG analyses demonstrated that the validated targets of

PVT1 were significantly enriched in several pathways, including the GnRH signaling pathway, the Cytokine-cytokine receptor interaction pathway, the Inflammatory mediator regulation of TRP channels pathway, and the Neuroactive ligand-receptor interaction pathway. **Conclusion:** PVT1 may serve as a potential biomarker associated with the progression and prognosis of human cancers.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

As the technology of RNA deep sequencing (RNA-seq) is being widely used, there is a deeper appreciation for the transcriptome of organisms. According to the differences in length, non-coding RNA (ncRNA) can commonly be classified into two categories: transcripts less than 200 nucleotides are usually accepted as short/small RNAs including miRNAs, siRNAs, piRNAs and those longer than 200 nucleotides are classified as long non-coding RNAs (lncRNAs) [1]. ncRNA, a class of RNA that does not encode proteins, was thought to be transcription noise or evolutionary junk in the past [2, 3]. However, multiple evidence have suggested that ncRNAs played important roles in a variety of biological processes. lncRNAs act as a regulator of gene expression and are closely associated with the development of many diseases, especially malignant tumors [4-7].

The human Plasmacytoma variant translocation 1 gene (PVT1) is an RNA gene homologous to the mouse PVT1 to the lncRNA class. This gene locus was first discovered in mouse plasma in the 1980s, and the strongest evidence for frequent variant translocation was found in plasmacytoma [8, 9]. Around the same time, studies showed that the PVT1 gene locus also underwent a variant translocation in human Burkitt's lymphomas [10, 11]. The PVT1 gene is located on chromosome 8, a recognized cancer risk locus shared with the well-known MYC oncogene_ENREF_12, suggesting that PVT1 is a potential oncogene [12]. *In vitro* experiments have demonstrated that PVT1 could promote proliferation, migration and invasion of cancer cells. Studies also observed that the level of PVT1 expression was higher in tumor tissues than in adjacent non-cancerous tissues and correlated with a poor prognosis [13, 14].

The evidence above revealed that PVT1 was involved in tumor progression. In addition, a previous meta-analysis had shown that the overexpression of PVT1 was significantly correlated with the prognosis and lymph node metastasis of cancers [15]. However, the sample size was limited, and the association of PVT1 with other clinicopathological parameters was not investigated in the study. Accordingly, we reviewed the pertinent literature and searched The Cancer Genome Atlas (TCGA) database to conduct the current study to exploring the clinicopathological and prognostic value of PVT1 in patients with various types of cancer. We also investigated the potential target genes of PVT1 through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, and the possible mechanisms of action of PVT1 in tumor progression are discussed in this article.

Materials and Methods

Meta-analysis

Literature review and selection

We conducted a search of the English and Chinese medical literature in PubMed, ISI Web of Science, EMBASE, Science Direct, Wiley Online Library, WanFang, China National Knowledge Infrastructure (CNKI), and Chinese VIP to identify all publications related to PVT1 in cancer patients. Based on the purpose of the study, the search strategy combined the terms "long non-coding RNA PVT1" OR "PVT1" OR "plasmacytoma variant translocation." We also checked for related comments and references by other means. The closing date of our search was July 4, 2017.

The studies eligible for our meta-analysis were evaluated by two independent investigators (RQ He and MJ Qin) with the same multi-step process. Initially, the titles and abstracts were read to exclude the

ineligible studies, which were either irrelevant, duplicate texts, reviews or case reports. Next, the full-text of the remaining studies were further checked by the same investigators individually. Finally, any disputes were addressed by a third reviewer (G Chen). Studies were considered eligible if they met the following inclusion criteria: (i) The clinical samples were gathered from tumor tissues; (ii) The correlation between PVT1 and survival data was studied and the PVT1 levels were measured by quantitative real-time PCR (qRT-PCR); and (iii) The studies should provide sufficient data to determine the hazard ratios (HRs) value and its 95% confidence interval (95% CI). The exclusion criteria were as follows: (i) Duplicated studies; (ii) Studies with insufficient data to calculate the HR value; (iii) Studies of animals or cell lines; and (iv) Reviews, letters, case reports and conference articles. If the survival analysis showed in the article were insufficient to calculate the HR value, we attempted to contact the author to obtain the original survival data.

Data extraction

The following key information was extracted from all eligible studies: the first author's name, publication year, cancer type, country, sample size, assay method, the criterion for dividing PVT1 into high and low groups, follow-up times, prognostic data, histological differentiation, tumor (T) classification, lymph node (N) classification, metastasis (M) classification and TNM stages. Engauge Digitizer 4.1 (<http://digitizer.sourceforge.net/>) and Jayne F Tierney's publication [16] were used to extract the HR and 95% CI indirectly from Kaplan-Meier survival curves if the articles did not provide complete survival data.

Quality assessment

The Newcastle-Ottawa Scale (NOS) [17] was used to evaluate the methodological quality of the selected studies by two investigators (RQ He and MJ Qin) independently. Each study was scored based on the following three part system: (i) selection, 0-4; (ii) comparability section, 0-2; and (iii) outcome section, 0-3. The score was no more than 9 points in total, and a score ≥ 6 indicated the good quality of the study.

Statistical analysis

The information extracted from eligible studies was analyzed with STATA 12.0 software. The survival data were evaluated by HR and 95%CI; PVT1 overexpression pointed to a poor prognosis if a pooled HR >1 was detected. The odd ratio (OR) and 95%CI were calculated to analyze the association of human cancer with the clinicopathological parameters, which included gender, histological differentiation, tumor (T) classification, lymph node (N) classification, metastasis (M) classification and TNM stages. In addition, we performed subgroup analysis based upon the tumor types and the sources of overall survival data. Cochran's Q test and the I^2 index were used to estimate the potential heterogeneity among the selected studies, $P < 0.05$ or $I^2 > 50\%$ were considered statistically significant. If there was no significant heterogeneity ($P > 0.05$), a fixed-effect model was used to calculate the HR values; otherwise, a random-effect model would be adopted. Finally, Begg's test was applied to evaluate the publication bias (a two-sided $P < 0.05$ was considered to be statistically significant).

Analysis of the level of PVT1 expression in all cancers based upon TCGA data

The level of PVT1 expression and overall survival data in the TCGA database were extracted from the OncoPrint website (<http://www.oncoprint.org/>) [18]. The experimental data were divided into two groups of high and low median level of PVT1 expression. The Cox proportional hazards model of SPSS 22.0 was used to estimate the influence of PVT1 overexpression on survival. We also obtained the level of PVT1 expression across all tumor samples and paired adjacent normal tissues from FireBrowse (<http://firebrowse.org/>) and GEPIA (<http://gepia.cancerpku.cn/>), an interactive web based TCGA database. Bar diagrams of PVT1 expression in cancers were plotted with Microsoft Excel 2003.

GO and KEGG Pathway analysis of the validated target genes of PVT1

We used TANRIC (http://ibl.mdanderson.org/tanric/_design/basic/index.html) [19] based upon the TCGA dataset and MultiExperiment Matrix (<http://biit.cs.ut.ee/mem/>) based upon the ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress/>) to determine the potential targets of PVT1 in human cancers. Venny diagram (<https://omictools.com/venny-tool>) was used to identify those genes overlapping the two online platforms. To confirm the most significant processes and signaling pathways of PVT1 in human cancers, Gene ontology (GO) [20] enrichment analysis was conducted with DAVID Bioinformatics Resources 6.8

(<https://david.ncifcrf.gov/>), and visualized with R version 3.3.3. The pathway enrichment analysis was carried out with Kyoto Encyclopedia of Genes and Genomes (KEGG) [21] _ENREF_21 data, which were obtained from DAVID Bioinformatics Resources 6.8. The network of PVT1 and their related genes were visualized using the Cytoscape 3.5.1 software with the Bingo plug-in.

Results

Meta-analysis

Literature selection and summary of the study characteristics

We conducted a search published studies of the association of PVT1 expression and prognosis of cancer patients (Fig. 1). A total of 722 potential studies were identified after the primary search, of which 42 studies were considered eligible after title and abstract screening. Next, we examined the full text of the remaining articles. Ultimately, 19 studies (n=2006) were included in our analysis and the main characteristics are shown in Table 1. The follow-up times ranged between 30 and 147.6 months. All selected studies investigated the association of PVT1 and survival analysis, including overall survival (OS), disease-specific survival (DSS), disease-free survival (DFS), relapse-free survival (RFS) or progression-free survival (PFS); 8 studies explored the association between PVT1 and histological differentiation, 14 for TNM stage, 5 for T classification, 11 for N stage, 7 for M classification and 15 for Gender (shown in Table 2).

Fig. 1. Flow diagram of the search and selection of study patients.

Correlation of lncRNA PVT1 with the clinicopathological characteristics of the study patients

As shown in Table 2, we discovered that high PVT1 level was remarkably related to histological differentiation (OR =1.63, 95%CI: 1.21-2.19, P=0.001; I² = 37.6%, and P=0.129) (Fig. 5A), TNM stages (OR =2.76, 95%CI: 2.18-3.50, P<0.01; I² = 41.2%, and P=0.054) (Fig. 5B), T stages (OR =5.08, 95%CI: 2.30-11.22, P<0.01; I² =69.3, and P=0.011) (Fig. 5C), positive lymph node metastasis (OR =2.14, 95%CI: 1.18-3.91, P=0.013; I² = 77.1% and P<0.01) (Fig. 6A). However, no significant association was found for distant metastasis (OR =2.43, 95%CI: 0.96-6.14, P=0.061; I² =54%, and P=0.042) (Fig. 6B) and gender (OR = 1.06, 95%CI: 0.84-1.34, P=0.611; I² = 0.0%, P=0.833) (Fig. 6C). The above results indicated that tumors with high PVT1 levels appeared to exhibit aggressive biological behavior.

Correlation of lncRNA PVT1 expression and survival

We performed an analysis of the association of PVT1 expression and OS based upon the outcomes of the 19 selected studies and TCGA database, which revealed that overexpression of PVT1 was significantly associated with a poor prognosis. A random-effect model was chosen to combine the HR due to high heterogeneity (I² =62.5%, P =0.00), the HR for OS was 1.31 (95% CI: 1.19-1.43, P<0.01) (Fig. 2). The pooled HR for DFS and RFS were 1.78 (95% CI: 1.45-2.17, P<0.01, fixed-effect model) with low heterogeneity (I² =0.0%, P=0.755) and 1.90 (95% CI: 1.31-2.75, P<0.01, fixed-effect model) with low heterogeneity (I² =0.0%, P=0.379), respectively (Fig. 4).

Table 1. Main characteristics of the selected studies. N (M/F): number (male/female); HR: hazard ratio; CI: confidence interval; GC: gastric carcinoma; OS: osteosarcoma; ESCC: esophageal squamous cell carcinoma; SCLC: small cell lung cancer; OC: ovarian cancer; CC: cervical cancer; NSCLC: non-small cell lung cancer; PDAC: pancreatic (ductal) adenocarcinoma; HCC: hepatocellular carcinoma; CRC: colon rectal carcinoma; OS: overall survival; DSS: disease-specific survival; DFS: disease-free survival; RFS: relapse-free survival; PFS: progression-free survival; NR: not report; *: approximate times extracted from survival curve

First author	Year	Country	Cancer types	N(M/F)	High expression	Follow-up (Months)	Outcome	HR (95%CI)	Quality score
Wu D [41]	2017	China	NSCLC	31(22/9)	NR	80*	OS	2.67(0.87-8.23)	8
Xu M [33]	2017	China	GC	190	by the mean value	85	DFS DSS	1.75 (1.25-2.56) 1.64 (1.15-2.39)	8
Song J [34]	2017	China	OS	46(27/19)	>Median expression for PVT1	72*	OS	2.43(1.22-4.80)	8
Li P [28]	2017	China	ESCC	104(24/80)	median level of PVT1	90*	OS DFS	2.75(1.35-5.59) 2.15(1.19-3.89)	8
Li Q [39]	2016	China	CRC	30(15/15)	NR	30	OS	2.02(1.07-3.83)	7
Huang C [27]	2016	China	SCLC	120(74/46)	median expression level of all samples	96*	OS	1.78(1.08-2.94)	8
Zhou Q [32]	2016	China	OS	53	NR	60*	OS	2.57(1.25-5.27)	7
Liu Y [29]	2016	China	OC	85(56/29)	≥median level 3.53	60*	OS	3.01(1.57-6.67)	8
Iden M [13]	2016	United State	CC	127	Median PVT1 expression level	NR	OS	2.02(1.07-3.82)	7
Wan L [14]	2016	China	NSCLC	100(62/43)	Median expression :fold-change≥3.0	36*	OS PFS	2.40(1.21-4.90) 2.51(1.58-3.98)	8
Chen J [26]	2016	China	GC	187(144/43)	NR	85	OS DFS	1.46(1.00-2.11) 1.43(1.00-2.06)	7
Huang C [54]	2015	China	PDAC	85(56/29)	Expression ratio≥3.53	60*	OS	3.01(1.57-6.67)	8
Yuan C [30]	2015	China	GC	111(70/41)	median value 5.22-fold	48	OS DFS	2.28(1.05-4.93) 2.21(1.11-4.39)	8
Cui D [55]	2015	China	NSCLC	108(69/39)	>Median level	38*	OS DFS	1.72(1.14-3.25) 1.98(1.16-4.35)	8
Ding C [56]	2015	China	HCC	214(195/19)	Above the cut-off	124	OS RFS	1.21(0.73-1.99) 1.65(1.02-2.68)	8
Kong R [36]	2015	China	GC	80(48/32)	>Median expression for PVT1	38*	OS DFS	2.03(1.07-4.10) 2.22(1.13-4.34)	8
Takahashi Y [22]	2014	Japan	CRC	164(97/67)	Above the 20 percentile value	147.6	OS	2.53(1.15-10.75)	8
Yang Y [57]	2014	China	NSCLC	82((49/33)	>Median expression level for PVT1 2.87)	60*	OS	3.27(2.18-6.94)	8
Wang F [23]	2014	China	HCC	89(73/16)	Above the cut-off value	50*	OS RFS	2.44(1.23-4.83) 2.32(1.30-4.14)	8

Table 2. Combinations of data evaluating the associations of PVT1 with the clinicopathological characteristics of the study patients

Clinicopathological parameters	Studies(n)	Test		Control		model	OR (95%CI)	Test for heterogeneity	
		Event	Total	Event	Total			Q	P
Histological differentiation (Poorly/Well-Moderately)	8(1050)	239	665	106	385	Fixed	1.63(1.21-2.19)	37.6%	0.129
TNM state(III-IV/I-II)	14(1439)	539	889	197	550	Fixed	2.76(2.18-3.50)	41.2%	0.054
T classification (T3-T4/T1-T2)	5(469)	174	282	47	187	random	5.08(2.30-11.22)	69.3%	0.011
N classification (N1-N3/N0)	11(1158)	435	718	196	440	random	2.14(1.18-3.91)	77.1%	<0.01
M classification (M1/M0)	7(855)	69	518	18	337	random	2.43(0.96-6.14)	54%	0.042
Gender(Male/Female)	15(1552)	645	976	356	576	Fixed	1.06(0.84-1.34)	0.0%	0.833

Fig. 2. Meta-analysis of the selected studies and TCGA data estimating the association of PVT1 with the patients' overall survival (OS) ($P < 0.01$, random effect model).

Fig. 3. Subgroup analyses of the overall survival (OS) based upon the tumor type ($P < 0.01$, fixed effect model).

Subgroup analysis
Considering the existence of heterogeneity in the studies, two subgroup analyses were conducted based upon the sources of survival data and the type of tumors. In the subgroup analysis for overall survival, we found that high expression of PVT1 was markedly associated with a worse overall survival in publications (HR = 2.03, 95%CI: 1.76-2.35, $P < 0.01$) and TCGA (HR = 1.09, 95%CI: 1.03-1.16, $P < 0.01$) (Fig. 2). Because the studies of colorectal cancer, prostate cancer, ovarian cancer, cervical cancer, and esophageal squamous cell carcinoma were

Fig. 4. Meta-analysis of selected studies estimating the association of PVT1 with Relapse-free Survival (RFS) and Disease-free Survival (DFS) ($P < 0.01$, fixed effect model).

Fig. 5. Meta-analysis estimating the correlation between PVT1 and clinicopathological parameters in cancer patients. A, histological differentiation ($P < 0.01$, fixed effect model); B, TNM stage ($P < 0.01$, fixed effect model); C, T classification ($P < 0.01$, random effect model);

single studies, we classified them as others. The same results were identified in the subgroup analysis based upon the tumor type (lung cancer (HR = 2.17, 95%CI: 1.65-2.85, $P < 0.01$), hepatocellular carcinoma (HR = 1.55, 95%CI: 1.03-2.32, $P = 0.035$), gastric cancer (HR = 1.66, 95%CI: 1.23-2.24, $P < 0.01$), osteosarcoma (HR = 2.50, 95%CI: 1.52-4.10, $P < 0.01$), colorectal cancer (HR = 2.14, 95%CI: 1.23-3.71, $P < 0.01$) and others including pancreatic cancer, ovarian cancer, cervical cancer, esophageal squamous cell carcinoma (HR = 2.62, 95%CI: 1.85-3.70, $P < 0.01$)) (Fig. 3). No heterogeneity was noted among these studies.

Publication bias

Begg's funnel plots were applied to assess the publication bias in our study. No publication bias was found in the studies estimating the associations of PVT1 with clinicopathological features and DFS. With regard to OS, a publication bias was not observed in the group derived from TCGA ($P = 0.651$). However, an asymmetric distribution of Egger's funnel plot appeared in the group of studies ($P = 0.01$); therefore,

Fig. 6. Meta-analysis estimating the correlation between PVT1 and clinicopathological parameters in cancer patients. A, N classification ($P=0.013$, random effect model); B, M classification ($P=0.061$, random effect model); C, dender ($P=0.611$, fixed effect model).

a trim-and-fill method was used to adapt the publication bias. The meta-analysis did not engender conflicting conclusions whether the trim-and-fill method was used or not, suggesting that the results of our study were statistically stable (Fig. 7-9).

TCGA data extraction

Based upon the results obtained from FireBrowse, PVT1 was highly expressed in 25 of the 37 tumor tissues except for the remaining 12 with missing data (Fig. 10). As shown in Fig. 11 derived from GEPIA, PVT1 was highly expressed in 22 types of cancers and was weakly expressed in 9 tumor tissues.

Function analysis of PVT1-related genes in human tumors

To explore the potential mechanism of action of PVT1, a total of 462 target genes were identified with TANRIC and MEM. GO and KEGG analyses were performed. The validated target genes for PVT1 enriched GO terms as follows: (i) biological processes (BPs): positive regulation of cytosolic calcium ion concentration, positive regulation of gene expression, chemical synaptic transmission, for example; (ii) cellular components (CCs): plasma membrane, integral component of plasma membrane, neuronal cell body, membrane, integral component of membrane, for example; and (iii) molecular factors (MFs): calcium ion binding, anion: anion antiporter activity, phosphatidylinositol phospholipase C activity, for example. Furthermore, KEGG enrichment

Fig. 7. Funnel plots (Begg's method) of potential publication bias in the selected studies. A, overall survival (OS) of study patients ($P < 0.01$); B, overall survival (OS) in TCGA data ($P = 0.651$); C, Disease-free Survival (DFS) ($P = 0.188$).

Fig. 8. Funnel plots (Begg's method) of potential publication bias in the selected studies. A, Gender ($P = 0.729$); B, histological differentiation ($P = 0.138$); C, TNM stage ($P = 0.25$).

analysis showed that PVT1 may play a pivotal role in cancers via multiple pathways including the GnRH signaling pathway, Cytokine-cytokine receptor interaction pathway, Inflammatory mediator regulation of TRP channels pathway, and Neuroactive ligand-receptor interaction pathway (Tables. 3-4 and Fig. 12-15).

Discussion

Numerous studies have shown that a high level of expression of PVT1 was significantly associated with the clinical prognosis and other clinicopathological parameters in cancer patients [22, 23]. Review articles have also summarized the critical role that PVT1 may play in the progression of a wide range of cancers [24, 25]. A meta-analysis by Liu et al. also suggested that the up-regulation of PVT1 predicted lymph node metastasis and a worse OS in

cancer patients, although the association between PVT1 and other clinicopathological parameters was not discussed [15]_ENREF_15. In addition, other publications since 2015 have continued to investigate the association between PVT1 expression and the prognosis of cancer patients [13, 14, 26-35]. To reach a more convincing conclusion and explore the potential mechanism of action of PVT1 in tumors, we performed the current study by merging the results of published studies and TCGA survival data and conducting GO and KEGG analyses.

The current meta-analysis of 2006 patients from 19 eligible studies and 7966 patients from TCGA explored the associations between high expression of PVT1 and the prognosis, as well as clinicopathological features in cancer patients. To the best of our knowledge, our study is the most comprehensive analysis to date. We evaluated the quality of all the selected studies by NOS, and a trim-and-fill test was used to examine the publication bias. If the p value of Begg's test was less than 0.05, we also checked the reliability of the results by sensitivity analysis. Our results showed that the high expression of PVT1 was apparently related to worse clinical outcomes, including OS was 1.31 (95% CI: 1.19-1.43), DFS (HR=1.78, 95%CI: 1.45-2.17), and RFS (HR=1.90, 95% CI: 1.31-2.75). For the clinicopathological features of patients with cancers, our study indicated that high PVT1 was significantly associated with the aggression of cancers, including histological differentiation (OR=1.63, 95%CI: 1.21-2.19), TNM stages (OR =2.76, 95%CI: 2.18-3.50), T stages (OR =5.08, 95%CI: 2.30-11.22) and lymph node metastasis (OR =2.14, 95%CI: 1.18-3.91). A subgroup analysis suggested that high PVT1 expression is significantly correlated with overall survival, both in the publications (HR =2.03, 95%CI: 1.76-2.35) and TCGA (HR=1.09, 95%CI: 1.03-1.16). Simultaneously, subgroup analysis also showed that PVT1 was significantly associated with all types of cancer. PVT1 appears to be a potential biomarker reflecting the progression and prognosis of cancer based upon the results of our study. However, rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), and stomach adenocarcinoma (SKAD) in the TCGA dataset were correlated with a good prognosis although the results were not statistically significant; their corresponding HR values were 0.786 (95%CI: 0.379-1.629, P= 0.517) , 0.92 (95%CI: 0.742-1.141, P= 0.449) and 0.912 (95%CI: 0.702-1.183, P= 0.486) , respectively. We reviewed the relevant studies and found that the high PVT1 level was associated with worse outcomes in gastric cancer and colorectal cancer [22, 26, 30, 33, 36] and that there was no corresponding report for melanoma. The inconsistent results in the TCGA and literature studies in these tumors may be caused by sampling error and publication bias. Additional studies are needed to confirm the association between PVT1 and prognosis in different types of cancer.

In the current meta-analysis, significant heterogeneity was present in the studies with OS (P<0.05) when combining the HR values from publications. Heterogeneity also emerged in the studies of the clinicopathological features, including T classification, lymph node metastasis and M classification (P<0.05). Considering that the presence of heterogeneity may

Fig. 9. Funnel plots (Begg's method) of potential publication bias in the selected studies. A, T classification (P=0.05); B, N classification (P=0.815); C, M classification (P=0.368).

Fig. 10. PVT1 expression profile across tumor samples and adjacent normal tissues from FireBrowse (box plot).

Fig. 11. PVT1 expression profile across tumor samples and paired normal tissues from GEPIA (barplot). The height of the bar represents the median expression of certain tumor types or normal tissues. A, the level of PVT1 expression was higher in tumor than in adjacent normal tissue; B, the level of PVT1 was lower in tumor than in adjacent normal tissue.

influence the results of the meta-analysis, we have treated the heterogeneity carefully [37]. ENREF_37 with a random effect model to reduce the effect of heterogeneity on the merged result in our study. A publication bias was also noted in studies with OS data ($P=0.01$) as

Table 3. GO functional annotation of the most significant related targets of PVT1

GO ID	GO term	Count (%)	Gene symbol	P
Biological process				
GO:0007204	positive regulation of cytosolic calcium ion concentration	19	KNG1, PTGER3, CCKBR, SWAP70, OPRL1, ESR1, LPAR3, CXCR3, etc	7.60E-09
GO:0010628	positive regulation of gene expression	19	E2F1, NOS1AP, CSF1, PIK3CD, HFE, PTPN22, HCFC1, LEF1, MAPK11, etc	1.25E-04
GO:0007268	chemical synaptic transmission	18	SNCG, MPZ, NRXN2, OPRL1, PCDHB3, CPNE6, CACNB1, PCDHB2, etc	1.33E-04
GO:0007155	cell adhesion	24	IBSP, ACHE, ICAM4, PCDHB3, LRRN2, MYBPC3, PCDHB2, ACTN2, etc	0.001469
GO:0006954	inflammatory response	21	CIITA, KNG1, IL18RAP, PTGER3, TNFRSF25, AIF1, IL1RL2, CSF1, etc	0.001613
GO:0002230	positive regulation of defense response to virus by host	5	LILRB1, IL12RB1, IL23A, PTPN22, APOBEC3G	0.002043
GO:0043401	steroid hormone mediated signaling pathway	7	ESRRA, NR1I2, THRA, ESR1, LEF1, HNF4G, NR2C1	0.00306
GO:0070588	calcium ion transmembrane transport	10	ATP2B2, TRPV1, ATP2A1, CACNB1, CACNG4, CACNG2, CACNB4, etc	0.003213
GO:0060041	retina development in camera-type eye	7	MAX, TUB, ACHE, CLCN2, LHX2, NTRK2, SLC4A5	0.003342
GO:0007202	activation of phospholipase C activity	5	S1PR4, AVPR1A, ADRA1A, LPAR1, PLCB2	0.003857
Cellular component				
GO:0005886	plasma membrane	163	F2RL2, ADCY2, CROCC, EFNA3, SLC9A2, LPAR3, SLC22A11, LPAR1, etc	5.18E-12
GO:0005887	integral component of plasma membrane	75	F2RL2, ADCY2, MSR1, TRPV1, EFNA3, LPAR3, SLC22A11, LPAR1, etc	1.57E-10
GO:0043025	neuronal cell body	20	UBXN1, SNCG, CPLX2, TRPV1, HCFC1, LPAR1, TMPRSS5, ITPR3, etc	2.44E-04
GO:0016020	membrane	77	ADCY2, MSR1, QPCTL, AP1G2, PKMYT1, VPS53, RPS3, IGHMBP2, etc	7.13E-04
GO:0016021	integral component of membrane	155	F2RL2, ADCY2, QPCTL, B3GALT4, SLC9A2, SLC22A11, GDAP1 L1, etc	0.001083
GO:0005929	cilium	10	SMO, ATP2B2, TUB, EVC, FAM49B, CCDC40, ADCY10, ATP6 V1D, ARL3, etc	0.011024
GO:0031095	platelet dense tubular network membrane	3	ATP2A1, ITPR3, ITPR2	0.018633
GO:0005891	voltage-gated calcium channel complex	4	CACNB1, CACNG4, CACNG2, CACNB4	0.031914
GO:0009897	external side of plasma membrane	11	NCAM1, LILRB1, ECE1, IL12RB1, CD80, TRPV1, TFR2, TNFRSF13B, etc	0.034066
GO:0032580	Golgi cisterna membrane	6	GALNT3, GOLGA8B, FUT7, ST3GAL4, GOLIM4, HMP19	0.036364
Molecular function				
GO:0005509	calcium ion binding	33	GALNT3, S100A3, ENPP1, MASP1, AIF1, KCNIP2, ATP2B2, MMP20, etc	9.81E-04
GO:0015301	anion:anion antiporter activity	5	SLC22A7, SLC22A8, SLC26A10, SLC26A1, SLC4A5	0.002259
GO:0004435	phosphatidylinositol phospholipase C activity	5	PLCL2, F2RL2, CCKBR, PLCB1, PLCB2	0.004141
GO:0005262	calcium channel activity	7	TRPV1, CACNG4, CACNG2, CACNB4, ITPR3, PKDREJ, TRPM2	0.00768
GO:0004872	receptor activity	13	AMHR2, IL18RAP, LILRA2, TNFRSF25, LRRN2, SIGLEC7, TNFRSF13B, etc	0.008029
GO:0003707	steroid hormone receptor activity	6	ESRRA, NR1I2, THRA, ESR1, HNF4G, NR2C1	0.012336
GO:0015347	sodium-independent organic anion transmembrane transporter activity	4	SLC22A7, SLC22A8, SLC22A11, SLC05A1	0.014394
GO:0003700	transcription factor activity, sequence-specific DNA binding	36	E2F1, POU6F1, RAI1, THRA, SPI1, HCFC1, ZBTB17, NR2C1, FOXH1, etc	0.01465
GO:0004887	thyroid hormone receptor activity	3	NR1I2, THRA, BRD8	0.015515
GO:0008307	structural constituent of muscle	5	OBSCN, MYBPC3, ACTN2, ASPH, NEBL	0.019768

Table 4. KEGG pathway enrichment analysis of PVT1 target genes

KEGG ID	KEGG term	Count (%)	Gene symbol	P
hsa04912	GnRH signaling pathway	11	PLD2, ADCY2, SOS2, MAPK11, MMP14, PLCB1, ITPR3, PLCB2, LHB, MAP2K6, ITPR2	5.66E-04
hsa04060	Cytokine-cytokine receptor interaction	18	AMHR2, IL18RAP, TNFRSF25, CTF1, CSF1, TNFRSF13B, IL21R, EDAR, CXCR3, CCL25, etc	9.55E-04
hsa04750	Inflammatory mediator regulation of TRP channels	11	ADCY2, TRPV1, PIK3CD, PLA2G6, MAPK11, PIK3R5, PLCB1, ITPR3, PLCB2, MAP2K6, ITPR2	0.001017607
hsa04080	Neuroactive ligand-receptor interaction	20	F2RL2, THRA, PTGER3, CCKBR, TRPV1, OPRL1, LPAR3, LPAR1, ADORA1, PLG, CRHR2, etc	0.001191953
hsa04630	Jak-STAT signaling pathway	13	CTF1, PIK3CD, IL21R, STAT2, IL23A, IL12RB1, SOS2, IL12A, PIK3R5, JAK3, IL5RA, AKT3, IL3RA	0.002092022
hsa04261	Adrenergic signaling in cardiomyocytes	13	ADCY2, PIK3CD, CACNB1, CACNG4, MAPK11, CACNB4, CACNG2, ATP2B2, ADRA1A, etc	0.002217002
hsa04725	Cholinergic synapse	11	ACHE, ADCY2, PIK3CD, PIK3R5, CHRNA6, GNG4, PLCB1, ITPR3, PLCB2, AKT3, ITPR2	0.002619522
hsa04380	Osteoclast differentiation	12	LILRB1, LILRA2, FCGR2C, CSF1, PIK3CD, LILRA5, SPI1, MAPK11, PIK3R5, AKT3, MAP2K6, STAT2	0.002839608
hsa04915	Estrogen signaling pathway	10	ADCY2, PIK3CD, SOS2, ESR1, PIK3R5, PLCB1, ITPR3, PLCB2, AKT3, ITPR2	0.003992286
hsa04062	Chemokine signaling pathway	14	ADCY2, PIK3CD, CXCR3, STAT2, CCL25, CCR3, SOS2, PIK3R5, JAK3, PLCB1, GNG4, XCR1, etc	0.006027544

shown by Begg's test and funnel plots. Hence, a trim-and-fill method was used to adapt the publication bias and the results were consistent with the previous one which suggested that the results of our study were statistically stable.

Fig. 12. Significantly enriched annotation of (Gene Ontology) GO categories and (Kyoto Encyclopedia of Genes and Genomes) KEGG pathways of potential mRNA targets of lncRNA PVT1 in cancer patients. A, biological processes (BP); B, cellular components (CC); C, molecular factors (MF); D, KEGG pathway.

Studies have shown that the level of PVT1 expression was clearly up-regulated in cancer tissues compared with paired adjacent tissues [31, 38-41], The same result was also detected in cell line samples *in vitro* [34, 42]. In recent years, studies on the mechanism of action of PVT1 in cancer have proliferated, and increasing evidence suggested that PVT1 could affect diverse biological behaviors of multiple tumors. Yan et al. observed that the expression of PVT1 was positively associated to miR-1207-5p, which promoted the breast cancer cell

Fig. 13. Gene Ontology (GO) analysis of the biological process (BP) category.

Fig. 14. Gene Ontology (GO) analysis of the cellular component (CC) category.

proliferation by targeting STAT6 [43]. Ma et al. revealed that PVT1 was highly expressed in glioma vascular endothelial cells, which increased the expression of Beclin1 and Atg7 by targeting miR-186, thereby promoting cell proliferation, migration and angiogenesis [44]. Lan et al. discovered that PVT1 acts as an endogenous sponge of miR-186-5p to reduce its inhibitory effect on yes-associated protein 1, promoting the tumorigenesis of hepatocellular carcinoma [45]. Although advances were made in the understanding of the role of PVT1 in tumors, its precise molecular mechanisms underlying its function are still unclear. Hence, we collected the validated targeting genes of PVT1 using MEM and TANRIC Platform and performed a comprehensive target gene network analysis. The GO and KEGG pathway analysis demonstrated that PVT1 may play a pivotal role in the human tumors via different

Acknowledgements

The study was supported by the funds of National Natural Science Foundation of China (NSFC 81560469, NSFC 81360327), Natural Science Foundation of Guangxi, China (2015GXNSFCA139009) and Guangxi Medical University Training Program for Distinguished Young Scholars (2017).

Disclosure Statement

The authors declare that they have no conflicts of interest.

References

- 1 Yan B, Wang Z: Long noncoding rna: Its physiological and pathological roles. *DNA Cell Biol* 2012;31:S-34-S-41.
- 2 Uchida S, Dimmeler S: Long noncoding rnas in cardiovascular diseases. *Circ Res* 2015;116:737.
- 3 Jian L, Jian D, Chen Q, Zhang L: Long noncoding rnas in atherosclerosis. *J Atheroscler Thromb* 2015;23:376.
- 4 Bhan A, Soleimani M, Mandal SS: Long noncoding rna and cancer: A new paradigm. *Cancer Res* 2017;77:3965.
- 5 Kocerha J, Dwivedi Y, Brennand KJ: Noncoding rnas and neurobehavioral mechanisms in psychiatric disease. *Mol Psychiatry* 2015;20:677-684.
- 6 Nobili L, Ronchetti D, Taiana E, Neri A: Long non-coding rnas in b-cell malignancies: A comprehensive overview. *Oncotarget* 2017;8:60605.
- 7 Tang J, Jiang R, Deng L, Zhang X, Wang K, Sun B: Circulation long non-coding rnas act as biomarkers for predicting tumorigenesis and metastasis in hepatocellular carcinoma. *Oncotarget* 2015;6:4505-4515.
- 8 Cory S, Graham M, Webb E, Corcoran L, Adams JM: Variant (6;15) translocations in murine plasmacytomas involve a chromosome 15 locus at least 72 kb from the c-myc oncogene. *EMBO J* 1985;4:675.
- 9 Webb E, Adams JM, Cory S: Variant (6 ; 15) translocation in a murine plasmacytoma occurs near an immunoglobulin | [kappa]| gene but far from the myc oncogene. *Nature* 1984;312:777-779.
- 10 Huppi K, Siwarski D: Chimeric transcripts with an open reading frame are generated as a result of translocation to the pvt-1 region in mouse b-cell tumors. *Int J Cancer* 1994;59:848.
- 11 Shtivelman E, Bishop JM: Effects of translocations on transcription from pvt. *Mol Cell Biol* 1990;10:1835.
- 12 Zeng C, Yu X, Lai J, Yang L, Chen S, Li Y: Overexpression of the long non-coding rna pvt1 is correlated with leukemic cell proliferation in acute promyelocytic leukemia. *J Hematol Oncol* 2015;8:1-6.
- 13 Iden M, Fye S, Li K, Chowdhury T, Ramchandran R, Rader JS: The lncrna pvt1 contributes to the cervical cancer phenotype and associates with poor patient prognosis. *PLoS One* 2016;11:e0156274.
- 14 Wan L, Sun M, Liu GJ, Wei CC, Zhang EB, Kong R, Xu TP, Huang MD, Wang ZX: Long noncoding rna pvt1 promotes non-small cell lung cancer cell proliferation through epigenetically regulating lats2 expression. *Mol Cancer Ther* 2016;15:1082-1094.
- 15 Liu FT, Xue QZ, Zhu ZM, Qiu C, Hao TF, Zhu PQ, Luo HL: Long noncoding rna pvt1, a novel promising biomarker to predict lymph node metastasis and prognosis: A meta-analysis. *Panminerva Med* 2016;58:160.
- 16 Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR: Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007;8:16.
- 17 Stang A: Critical evaluation of the newcastle-ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010;25:603.
- 18 Anaya J: Oncolnc: Linking tcga survival data to mrnas, mirnas, and lncrnas. *PeerJ Computer Science* 2016;2:e67.
- 19 Li J, Han L, Roebuck P, Diao L, Liu L, Yuan Y, Weinstein JN, Liang H: Tanric: An interactive open platform to explore the function of lncrnas in cancer. *Cancer Res* 2015;75:3728-3737.

- 20 Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G: Gene ontology: Tool for the unification of biology. *Nat Genet* 2000;25:25-29.
- 21 Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M: Kegg for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res* 2011;40:D109-D114.
- 22 Takahashi Y, Sawada G, Kurashige J, Uchi R, Matsumura T, Ueo H, Takano Y, Eguchi H, Sudo T, Sugimachi K, Yamamoto H, Doki Y, Mori M, Mimori K: Amplification of pvt-1 is involved in poor prognosis via apoptosis inhibition in colorectal cancers. *Br J Cancer* 2014;110:164-171.
- 23 Wang F, Yuan JH, Wang SB, Yang F, Yuan SX, Ye C, Yang N, Zhou WP, Li WL, Li W, Sun SH: Oncofetal long noncoding rna pvt1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing nop2. *Hepatology* 2014;60:1278-1290.
- 24 Colombo T, Farina L, Macino G, Paci P: Pvt1: A rising star among oncogenic long noncoding rnas. *Biomed Res Int* 2015;2015:304208.
- 25 Cui M, You L, Ren X, Zhao W, Liao Q, Zhao Y: Long non-coding rna pvt1 and cancer. *Biochem Biophys Res Commun* 2016;471:10-14.
- 26 Chen J, Li Y, Zheng Q, Bao C, He J, Chen B, Lyu D, Zheng B, Xu Y, Long Z, Zhou Y, Zhu H, Wang Y, He X, Shi Y, Huang S: Circular rna profile identifies circpvt1 as a proliferative factor and prognostic marker in gastric cancer. *Cancer Lett* 2017;388:208-219.
- 27 Huang C, Liu S, Wang H, Zhang Z, Yang Q, Gao F: Lncrna pvt1 overexpression is a poor prognostic biomarker and regulates migration and invasion in small cell lung cancer. *Am J Transl Res* 2016;8:5025.
- 28 Li PD, Hu JL, Ma C, Ma H, Yao J, Chen LL, Chen J, Cheng TT, Yang KY, Wu G: Upregulation of the long non-coding rna pvt1 promotes esophageal squamous cell carcinoma progression by acting as a molecular sponge of mir-203 and lasp1. *Oncotarget* 2017;8:34164-34176.
- 29 LIU Y, Quan X, Z, Qin X, M, Lan Y, L: Effect of neoadjuvant chemotherapy on difference of expression of lncrna in ovarian cancer patients. *Practical Journal of Cancer* 2016;31:384-386.
- 30 Yuan CL, Li H, Zhu L, Liu Z, Zhou J, Shu Y: Aberrant expression of long noncoding rna pvt1 and its diagnostic and prognostic significance in patients with gastric cancer. *Neoplasma* 2016;63:442-449.
- 31 Zhang S, Zhang G, Liu J: Long noncoding rna pvt1 promotes cervical cancer progression through epigenetically silencing mir-200b. *APMIS* 2016;124:649-658.
- 32 Zhou Q, Chen F, Zhao J, Li B, Liang Y, Pan W, Zhang S, Wang X, Zheng D: Long non-coding rna pvt1 promotes osteosarcoma development by acting as a molecular sponge to regulate mir-195. *Oncotarget* 2016;7
- 33 Xu MD, Wang Y, Weng W, Wei P, Qi P, Zhang Q, Tan C, Ni SJ, Dong L, Yang Y: A positive feedback loop of lncrna-pvt1 and foxm1 facilitates gastric cancer growth and invasion. *Clin Cancer Res* 2017;23:2071.
- 34 Song J, Wu X, Liu F, Li M, Sun Y, Wang Y, Wang C, Zhu K, Jia X, Wang B, Ma X: Long non-coding rna pvt1 promotes glycolysis and tumor progression by regulating mir-497/hk2 axis in osteosarcoma. *Biochem Biophys Res Commun* 2017
- 35 Wang BJ, Ding HW, Ma GA: Long noncoding rna pvt1 promotes melanoma progression via endogenous sponging mir-26b. *Oncol Res* 2017
- 36 Kong R, Zhang EB, Yin DD, You LH, Xu TP, Chen WM, Xia R, Wan L, Sun M, Wang ZX, De W, Zhang ZH: Long noncoding rna pvt1 indicates a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically regulating p15 and p16. *Mol Cancer* 2015;14:82.
- 37 Munafo MR, Flint J: Meta-analysis of genetic association studies. *Trends Genet* 2004;20:439-444.
- 38 Ding J, Li D, Gong M, Wang J, Wu T, Wang C: Expression and clinical significance of long non-coding rna-pvt1 in human gastric cancer. *Onco Targets Ther* 2015;35:1615-1617.
- 39 Li Q, Dai Y, Wang F, Hou S: Differentially expressed long non-coding rnas and the prognostic potential in colorectal cancer. *Neoplasma* 2016;63:977.
- 40 Liu F, Dong Q, Huang J: Overexpression of lncrna pvt1 predicts advanced clinicopathological features and serves as an unfavorable risk factor for survival of patients with gastrointestinal cancers. *Cell Physiol Biochem* 2017;43:1077-1089.
- 41 Wu D, Li Y, Zhang H, Hu X: Knockdown of lncrna pvt1 enhances radiosensitivity in non-small cell lung cancer by sponging mir-195. *Cell Physiol Biochem* 2017;42:2453-2466.
- 42 Zhuang C, Li J, Liu Y, Chen M, Yuan J, Xing F, Zhan Y, Li L, Lin J, Zhou Q: Tetracycline-inducible shrna targeting long non-coding rna pvt1 inhibits cell growth and induces apoptosis in bladder cancer cells. *Oncotarget* 2015;6:41194-41203.

- 43 Yan C, Chen Y, Kong W, Fu L, Liu Y, Yao Q, Yuan Y: Pvt1-derived mir-1207-5p promotes breast cancer cell growth by targeting stat6. *Cancer Sci* 2017;108:868-876.
- 44 Ma Y, Wang P, Xue Y, Qu C, Zheng J, Liu X, Ma J, Liu Y: Pvt1 affects growth of glioma microvascular endothelial cells by negatively regulating mir-186. *Tumour Biol* 2017;39:1010428317694326.
- 45 Lan T, Yan X, Li Z, Xu X, Mao Q, Ma W, Hong Z, Chen X, Yuan Y: Long non-coding rna pvt1 serves as a competing endogenous rna for mir-186-5p to promote the tumorigenesis and metastasis of hepatocellular carcinoma. *Tumour Biol* 2017;39:1010428317705338.
- 46 Schally AV: Hypothalamic hormones: From neuroendocrinology to cancer therapy. *Anticancer Drugs* 1994;5:115-130.
- 47 Stanislaus D, Pinter JH, Janovick JA, Conn PM: Mechanisms mediating multiple physiological responses to gonadotropin-releasing hormone. *Mol Cell Endocrinol* 1998;144:1.
- 48 Maggi R, Cariboni AM, Marelli MM, Moretti RM, André V, Marzagalli M, Limonta P: GnRH and gnrh receptors in the pathophysiology of the human female reproductive system. *Hum Reprod Update* 2016;22:358-381.
- 49 Millar RP: GnRH II and type II GnRH receptors. *Trends Endocrinol Metab* 2003;14:35-43.
- 50 Emons G, Schally AV: The use of luteinizing hormone releasing hormone agonists and antagonists in gynaecological cancers. *Hum Reprod* 1994;9:1364-1379.
- 51 Yano T, Pinski J, Radulovic S, Schally AV: Inhibition of human epithelial ovarian cancer cell growth *in vitro* by agonistic and antagonistic analogues of luteinizing hormone-releasing hormone. *Proc Natl Acad Sci U S A* 1994;91:1701-1705.
- 52 Kwok CW, Treeck O, Buchholz S, Seitz S, Ortmann O, Engel JB: Receptors for luteinizing hormone-releasing hormone (gnrh) as therapeutic targets in triple negative breast cancers (tnbc). *Target Oncol* 2015;10:365-373.
- 53 Rick FG, Schally AV: Bench-to-bedside development of agonists and antagonists of luteinizing hormone-releasing hormone for treatment of advanced prostate cancer. *Urol Oncol* 2015;33:270-274.
- 54 Huang C, Yu W, Wang Q, Cui H, Wang Y, Zhang L, Han F, Huang T: Increased expression of the lncrna pvt1 is associated with poor prognosis in pancreatic cancer patients. *Minerva Med* 2015;106:143.
- 55 Cui D, Yu CH, Liu M, Xia QQ, Zhang YF, Jiang WL: Long non-coding rna pvt1 as a novel biomarker for diagnosis and prognosis of non-small cell lung cancer. *Tumour Biol* 2016;37:4127-4134.
- 56 Ding C, Yang Z, Lv Z, Du C, Xiao H, Peng C, Cheng S, Xie H, Zhou L, Wu J, Zheng S: Long non-coding rna pvt1 is associated with tumor progression and predicts recurrence in hepatocellular carcinoma patients. *Oncol Lett* 2015;9:955-963.
- 57 Yang YR, Zang SZ, Zhong CL, Li YX, Zhao SS, Feng XJ: Increased expression of the lncrna pvt1 promotes tumorigenesis in non-small cell lung cancer. *Int J Clin Exp Pathol* 2014;7:6929.