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Differential Prognostic Impact of PTK6 Between Invasive Ductal Carcinoma and Special Histological Types in Triple-Negative Breast Cancer

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Abstract

Background

PTK6 is a prognostic biomarker in triple-negative breast cancer (TNBC), but its role across histological subtypes remains unclear. This study compared PTK6 expression and its prognostic impact between invasive ductal carcinoma of no special type (IDC) and special histological types (SHT) in TNBC.

Methods

A cohort of 192 TNBC patients (145 IDC, 47 SHT) was retrospectively analyzed. PTK6 expression was assessed by immunohistochemistry. Associations with clinicopathological features and survival (DFS, OS) were evaluated using chi-square tests, Kaplan-Meier curves, and Cox regression stratified by histological subtype.

Results

PTK6 positivity rates were comparable between IDC (55.2%) and SHT (46.8%, P = 0.318). In IDC, PTK6 positivity was significantly associated with lymph node metastasis (P < 0.01), distant metastasis/recurrence (P = 0.008), shorter DFS (P = 0.007) and OS (P = 0.009), and remained an independent prognostic factor for DFS (P = 0.047). In SHT, PTK6 positivity correlated only with lymph node metastasis (P = 0.002) but not with survival (DFS P = 0.980; OS P = 0.162).

Conclusion

PTK6 is a strong prognostic indicator in IDC but not in special histological types of TNBC. Histological subtyping should be incorporated when evaluating PTK6 as a biomarker or therapeutic target in TNBC.

Introduction

Triple-negative breast cancer (TNBC), comprising roughly 10% to 20% of all breast malignancies, is notoriously aggressive and carries a grim prognosis compared to other subtypes[1–3].Although cytotoxic chemotherapy and immune checkpoint blockade have advanced the therapeutic landscape, there remains an unmet need for robust prognostic biomarkers to guide clinical decision-making[4, 5].In this context, the KEYNOTE-355 trial provided pivotal evidence that combining pembrolizumab with chemotherapy confers a survival benefit specifically in patients with PD-L1-positive metastatic TNBC[6, 7]. Concurrently, novel antibody-drug conjugates (ADCs) directed against tumors with low HER2 expression have emerged as a promising strategy, expanding treatment options for this challenging disease[8–10].

TNBC is characterized by heterogeneity not only at the genomic level but also in its histological presentation, with diverse subtypes exhibiting markedly different clinical behaviors[11–13]. The most common form is invasive ductal carcinoma (IDC-NST), whereas special histological types (SHT)—including medullary, metaplastic, lobular, micropapillary, and apocrine carcinomas—demonstrate unique pathological features and prognoses[14–16]. Among these, TNBC-ILC surprisingly shows worse survival despite lower histological grade[17–19], and metaplastic carcinoma is notably aggressive and heterogeneous[20–22]. Hence, histological subtyping carries significant prognostic implications that should be integrated into clinical risk stratification[23, 24].

Protein tyrosine kinase 6 (PTK6), also referred to as breast tumor kinase (BRK), is a non-receptor tyrosine kinase that is aberrantly overexpressed in 40% to 70% of TNBC cases[25–27]. Functionally, PTK6 serves as a critical node in multiple oncogenic signaling cascades, driving tumor cell survival, epithelial–mesenchymal transition (EMT), and metastatic dissemination through the AKT, SRC, and EGFR pathways[28–31].Clinically, elevated PTK6 expression in TNBC has been consistently correlated with nodal metastasis and diminished overall survival, underscoring its prognostic relevance[32–34]. Beyond its cell-intrinsic effects, emerging evidence indicates that PTK6 actively modulates the tumor immune microenvironment; pharmacologic inhibition of PTK6 has been shown to enhance intratumoral infiltration of CD8 + T cells and natural killer (NK) cells, suggesting an immunomodulatory role [35–37]. Most notably, a recently developed proteolysis-targeting chimera (PROTAC) degrader, MS105, achieves specific PTK6 degradation and triggers apoptosis in breast cancer cells, demonstrating superior efficacy over conventional kinase inhibitors alone [38–40]. Collectively, these observations establish PTK6 as both a promising prognostic biomarker and an actionable therapeutic target in TNBC[41, 42].

Whether the prognostic value of PTK6 in TNBC is modified by histological subtype has not been previously addressed [43]. Given the marked biological and clinical disparities between IDC-NST and SHT with respect to their microenvironment, molecular alterations, and outcomes[14, 15, 20], we hypothesized a subtype-dependent role for PTK6. To explore this, we compared PTK6 expression and its prognostic impact between the two histological groups in 192 TNBC patients, employing chi-square tests, Kaplan–Meier curves, and Cox regression with subtype stratification [44].

Materials and methods

1. Study Design and Patient Cohort

This retrospective cohort study was conducted at Nanchang People's Hospital (formerly The Third Hospital of Nanchang). We reviewed the medical records and pathological specimens of patients who underwent mastectomy for invasive breast carcinoma between January 2013 and December 2018. Cases were included if they met the following criteria: (1) a definitive pathological diagnosis of invasive breast carcinoma; (2) confirmation of triple-negative breast cancer (TNBC) status via immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH), defined as negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2

(HER2); and (3) complete clinicopathological data and postoperative follow-up records. Exclusion criteria comprised bilateral breast cancer, presence of synchronous malignancies, receipt of preoperative radiotherapy, neoadjuvant chemotherapy, or endocrine therapy, and death from causes unrelated to breast cancer (e.g., accidents or acute illnesses) during the follow-up period.

From an initial pool of patients, 192 were ultimately enrolled in this study. Based on central pathological review, these cases were stratified into two distinct histological subgroups: 145 cases of invasive ductal carcinoma of no special type (IDC-NST) and 47 cases of special histological types (SHT), which included medullary, metaplastic, lobular, micropapillary, and apocrine differentiation. The date of surgery served as the baseline for survival calculations. Five-year overall survival (OS) and disease-free survival (DFS) were ascertained through telephone interviews or review of imaging records. All participants provided written informed consent prior to their inclusion in the study.

2. Histopathological and Immunohistochemical Evaluation

All surgically resected specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. For IHC analysis, 4-μm-thick sections were cut, deparaffinized in xylene, and rehydrated through a graded ethanol series. Antigen retrieval was performed by heating the sections in EDTA buffer (pH 9.0) using a high-pressure steam system for 3 minutes. After cooling to room temperature, endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide.

The immunostaining was performed using the EnVision two-step detection system. Primary antibodies against ER (clone SP1), PR (clone SP2), and HER2 (VENTANA anti-HER2/neu (4B5)) were procured from Fujian Maixin and Roche, respectively. For PTK6 detection, a primary rabbit polyclonal antibody (abs117941, Uning Wei) was diluted at 1:400 and applied to the sections overnight at 4°C. Following extensive washes with phosphate-buffered saline (PBS), the sections were incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody (MaxVision-HRP, Fuzhou Maixin) for 45 minutes at room temperature. The immune complexes were visualized using 3,3'-diaminobenzidine (DAB) as the chromogen, and the sections were counterstained with hematoxylin.

The interpretation of IHC results was performed by two experienced pathologists who were blinded to the patients' clinical data. PTK6 expression was semiquantitatively assessed using the immunoreactive score (IRS) system[45]. This score was calculated by multiplying the staining intensity (scored from 0 to 3 for negative, weak, moderate, and strong staining, respectively) by the percentage of positively stained tumor cells (scored from 0 to 4 for 0%, 1–10%, 11–50%, 51–70%, and 71–100%, respectively). The resulting scores ranged from 0 to 12, and a median score of 4.0 was used as the cut-off to define "PTK6-negative" (IRS 0–4) and "PTK6-positive" (IRS 5–12) groups.

(2)The interpretation of HER2 staining was according to the Breast Cancer HER-2 Detection Guidelines (2024 edition)[46]: 0: No membrane staining observed in any invasive cancer cell. 1+: Faint/incomplete membrane staining in any proportion of invasive cancer cells. 2+: Weak to moderate, complete but

heterogeneous membrane staining in > 10% of invasive cancer cells, or strong and complete membrane staining in ≤ 10% of invasive cancer cells. 3+: Strong and complete membrane staining in ≥ 10% of invasive cancer cells.

(3) IHC positive for ER and PR was defined as ≥ 1% tumor cell nuclei showing different degrees of staining. Negative ER and PR were defined as < 1% tumor cell nuclei with varying degrees of staining or no staining[47].

3.Fluorescence In Situ Hybridization (FISH) for HER2

For cases with equivocal HER2 IHC results (2+), FISH was performed to assess HER2 gene amplification. Briefly, 4-μm tissue sections were baked overnight at 60°C, deparaffinized, and pretreated with protease at 37°C. The sections were then denatured at 83°C and hybridized overnight at 42°C with a dual-probe set specific for the HER2 gene (orange signal) and the centromere of chromosome 17 (CEP17, green signal). After post-hybridization washes, the nuclei were counterstained with DAPI. Fluorescence signals were visualized under a fluorescence microscope, and the ratio of HER2 to CEP17 signals was calculated by counting at least 100 tumor cell nuclei. A ratio of ≥ 2.0 was interpreted as HER2 gene amplification (positive), while a ratio < 2.0 indicated no amplification (negative).

4. Statistical Analysis

All statistical analyses were conducted using SPSS version 22.0 (IBM Corp.) and GraphPad Prism version 9.0 (GraphPad Software). Continuous variables, such as PTK6 IRS scores, were compared between groups using the t-test (GraphPad). The relationships between PTK6 expression and clinicopathological parameters were evaluated using the Chi-square test, with continuity correction or Fisher's exact test applied where appropriate.

Survival curves for DFS and OS were constructed using the Kaplan-Meier method, and differences between the PTK6-positive and PTK6-negative groups were assessed using the log-rank test. To identify independent prognostic factors, a Cox proportional hazards regression model was employed. Variables found to be significant in the univariate analysis ($P < 0.1$) were subsequently included in the multivariate Cox model. A two-sided P-value of less than 0.05 was considered statistically significant for all tests.

Results

3.1 Baseline characteristics stratified by histological subtype

A total of 192 TNBC patients were included, comprising 145 (75.5%) with IDC-NST and 47 (24.5%) with SHT (e.g., medullary, metaplastic, lobular, micropapillary, and apocrine differentiation). As shown in Table

1, there were no significant differences between the two groups in age distribution, T stage, HER2-low expression, or PTK6 positivity (all P > 0.05). However, the IDC-NST group had a significantly higher rate of lymph node metastasis (57.2% vs. 34.0%, P = 0.006) and a greater proportion of high-grade (G3) tumours (72.4% vs. 34.0%, P < 0.01) compared with the SHT group.PTK6 staining is shown in Fig. 1.

Table 1
Clinicopathological characteristics of TNBC patients by histological subtype (IDC vs. SHT)

Clinicopathologic feature		**Cases(n)**	**Histological type**		**P Value**
			IDC	**SHT**	
Age(y)	<50	97(50%)	75(39%)	22(11%)	0.558[a]
	≥ 50	95(50%)	70(37%)	25(13%)	
Histological grading	I-II	71(37%)	40(21%)	31(16%)	<0.01[a]
	III	121(63%)	105(55%)	16(8%)	
HER2 Low Expression	Yes	92(38%)	71(37%)	21(11%)	0.609[a]
	No	100(62%)	74(39%)	26(14%)	
PTK6 Expression	Positive	102(31%)	80(31%)	22(31%)	0.318[a]
	Negative	90(69%)	65(69%)	25(69%)	
T stage	T1-T2	172(90%)	132(69%)	40(21%)	0.248[a]
	T3-T4	20(10%)	13(6%)	7(4%)	
N stage	N0	93(48%)	62(32%)	31(16%)	0.006[a]
	N1-N3	99(52%)	83(44%)	16(8%)	
Note:IDC represented invasive ductal Carcinoma; SHT represented special histological types carcinoma; HER N represented HER2 negative; HER L represented HER2 low expression of HER2;P represented P value.					
[a]Pearson Chi-Square					
[b]Continuity Correction					
[c]Fisher's Exact Test					

3.2 Association of PTK6 expression with clinicopathological features within each histological subtype

Table 2 presents the associations between PTK6 expression and clinicopathological variables in the IDC and SHT groups. In the IDC group, PTK6 positivity was significantly associated with lymph node

metastasis ($P < 0.01$), distant metastasis/recurrence ($P = 0.008$), and shorter survival ($P = 0.018$). In the SHT group, PTK6 positivity was only significantly associated with lymph node metastasis ($P = 0.002$). No other significant associations were observed in either subgroup (all $P > 0.05$).

Table 2
Association of PTK6 expression with clinicopathological features in IDC and SHT group

Clinicopathologic features	IDC		P	SHT		P
	-	+		-	+	
Age(y)			0.645[a]			0.861[a]
≥ 50	30	40		13	12	
<50	35	40		12	10	
Site			0.039[a]			0.391[a]
Left breast	42	38		14	15	
Right breast	23	42		11	7	
Histological grading			0.979[a]			0.351[c]
I-II	18	22		18	13	
III	47	58		7	9	
T stage			0.174[b]			0.524[b]
T1-T2	62	70		20	20	
T3-T4	3	10		5	2	
N stage			<0.01[a]			0.002[b]
N0	44	18		22	9	
N1-3	21	62		3	13	
HER2			0.542[a]			0.062[a]
N	35	39		17	9	
L	30	41		8	13	
D or R			0.008[b]			1[b]

Note:IDC represented invasive ductal Carcinoma; SHT represented special histological types carcinoma; HER2 N represented HER2 negative; HER2 L represented HER2 low expression of HER2;D or R distant represented metastasis or recurrence.N represented not detected.P represented P value.

[a]Pearson Chi-Square

[b]Continuity Correction

[c]Fisher's Exact Test

Clinicopathologic features	IDC		P	SHT		P
	-	+		-	+	
Yes	8	24		4	4	
No	59	56		21	18	
Survival time(y)			0.018[c]			0.414[b]
<5	1	11		0	2	
≥ 5	64	69		25	20	

Note:IDC represented invasive ductal Carcinoma; SHT represented special histological types carcinoma; HER2 N represented HER2 negative; HER2 L represented HER2 low expression of HER2;D or R distant represented metastasis or recurrence.N represented not detected.P represented P value.

[a]Pearson Chi-Square

[b]Continuity Correction

[c]Fisher's Exact Test

3.3 Impact of PTK6 expression on survival outcomes within each histological subtype

Survival analyses are presented in Fig. 2 in the IDC group (n = 145), PTK6-positive patients had significantly worse disease-free survival (DFS) than PTK6-negative patients (log-rank P = 0.007), and similarly, overall survival (OS) was significantly shorter in the PTK6-positive group (P = 0.009). The survival curves began to separate early during follow-up and remained divergent thereafter.

In the SHT group (n = 47), no significant difference was observed between PTK6-positive and PTK6-negative patients for either DFS (P = 0.980) or OS (P = 0.162)(as shown in Fig. 3). The two survival curves largely overlapped throughout the follow-up period, indicating that PTK6 expression did not affect survival outcomes in this subgroup.

3.4 Multivariate Cox regression analysis within each histological subtype

Univariate and multivariate Cox regression analyses were performed separately for the IDC and SHT groups, with results summarised in Table 3 and Table 4.

In the IDC group, univariate analysis showed that PTK6 positivity was significantly associated with worse disease-free survival (DFS, P = 0.011) and overall survival (OS, P = 0.033). N stage was also a significant predictor for DFS (P = 0.006), and age showed a borderline association with DFS (P = 0.049). In

multivariate analysis, N stage remained an independent prognostic factor for DFS (P = 0.047). No multivariate model was reported for OS.

Table 3
COX survival analysis of IDC group

parameter	DFS	P	OS	P
	HR(95%CI)		HR(95%CI)	
COX univariate survival analysis				
PTK6 expression	2.822 (1.272–6.259)	0.011	1.250(1.914–6.615)	0.033
HER2 expression	0.761(0.409–1.416)	0.389	1.421(0.493–4.097)	0.515
Histological type	1.269 (0. 688 – 2.340)	0.446	0.533(0.119–2.381)	0.410
breast	0.824(0.403–1.687)	0.597	1.296(0.455–3.695)	0.627
Age(y)	2.040(1.0034.147)	0.049	3.401(0.921–4.256)	0.066
Histological grading	1.162(0.836–1.615)	0.371	1.229(0.688–2.195)	0.485
T stage	1.344(0.774–2.335)	0.293	1.625(0.739–3.574)	0.227
N stage	3.215 (1.395–5.937)	0.006	6.568(0.893–8.315)	0.056
COX multifactor survival analysis				
N stage	2.261 (1.010–5.058)	0.047		

In the SHT group, univariate analysis revealed no significant association between PTK6 expression and DFS (P = 0.980) or OS (P = 0.474). None of the other variables reached statistical significance (all P > 0.05). Multivariate analysis was not performed due to the lack of significant predictors.

Table 4
COX survival analysis of SHT group

parameter	DFS	P	OS	P
	HR(95%CI)		HR(95%CI)	
COX univariate survival analysis				
PTK6 expression	1.018 (0.254–4.070)	0.980	0.639(0.019–2.554)	0.474
HER2 expression	0.166(0.020–1.349)	0.093	1.269(0.079–2.028)	0.866
Histological type	1.269 (0. 688 – 2.340)	0.446	0.533(0.119–2.381)	0.410
breast	0.502(0.101–2.490)	0.399	1.583(0.399–2.532)	0.745
Age(y)	0.865(0.216–3.459)	0.837	3.401(0.921–12.56)	0.483
Histological grading	0.499(0.175–1.422)	0.193	0.159(0.071–3.704)	0.554
T stage	0.970(0.741–1.836)	0.620	0.097(0.022–3.703)	1.826
N stage	2.209(0.552–8.838)	0.263	1.697(0.091–2.870)	0.436
COX multifactor survival analysis				

Discussion

The prognostic impact of PTK6 in TNBC proved to be highly contingent upon histological subtype in this cohort of 192 patients. Specifically, PTK6 positivity in the IDC-NST group was significantly correlated with lymph node metastasis, reduced DFS, and inferior OS, and independently predicted DFS on multivariable analysis[32–34]. Conversely, in the SHT group, although PTK6 expression again showed an association with nodal spread, it did not predict either DFS or OS, indicating that its prognostic value may be attenuated or superseded by subtype-specific biological determinants[43, 48].

These findings extend previous work on PTK6's overall prognostic impact in TNBC by introducing histological subtype as a stratifying factor[32, 33, 43]. The differential role may stem from biological differences[11, 14]. The IDC group typically has high proliferative activity and frequent EMT[23, 24]. PTK6 drives EMT via focal adhesion kinase phosphorylation and AKT activation[28–30], and also stabilizes SNAIL to repress E-cadherin[31, 49]. In immunocompetent mouse models, PTK6 inhibition (e.g., with P21d) increased CD8 + T cell and NK cell infiltration, reducing tumor growth[35–37]. In the aggressive, highly proliferative IDC group, both cell-autonomous and immune-modulatory pathways likely operate, making PTK6 a strong driver of poor outcomes[34, 42].

Conversely, the SHT group has distinct features that may override PTK6's impact. Metaplastic carcinoma often involves PI3K/AKT pathway alterations and mesenchymal differentiation that bypass PTK6 signaling[20, 21, 50]. TNBC-ILC shows low proliferation but distinct genetic drivers (ERBB2/ESRRA, DNA damage response) and poor chemotherapy response[17–19]. Medullary carcinoma, despite high grade,

often has dense tumor-infiltrating lymphocytes and favorable outcomes, where robust immune surveillance may overshadow PTK6 effects[14, 51, 52]. Thus, the absence of a PTK6-survival association in the SHT group is consistent with TNBC heterogeneity and underscores the need for subtype-specific analyses[12, 13, 53].

PTK6 positivity was associated with lymph node metastasis in both subgroups, suggesting a common role in promoting dissemination via p190RhoGAP, SRC, and SNAIL[31, 49, 54]. However, this did not translate into survival differences in the SHT group, possibly due to distinct metastatic patterns, undertreatment, or immune factors. A nationwide study found that only 40% of TNBC-ILC patients received adjuvant chemotherapy versus 59% of TNBC-NST patients, which may contribute to poor survival[18, 19].

Regarding HER2-low expression, now a therapeutic category after trastuzumab deruxtecan approval[8–10], we observed no significant difference in PTK6 positivity between HER2-low and HER2-negative tumors in either subgroup, and HER2-low status was not associated with survival. Meta-analyses show HER2-low confers only a modest survival advantage in TNBC[55–57]. Given that PTK6 is co-amplified with HER2 in some breast cancers and may cooperate with HER2[58–60], further studies should explore whether PTK6-positive/HER2-low TNBC represents a unique subgroup amenable to PTK6-targeted or HER2-directed therapies[38, 39, 61].

Limitations include single-center retrospective design, small SHT group sample size (n = 47) precluding analysis of individual rare subtypes[14, 20], lack of functional assays, and non-uniform treatment regimens. External validation is needed[62]. Strengths include being the first study to compare PTK6 prognostic value between the IDC group and the SHT group in TNBC, using stratified analyses, and employing routine immunohistochemistry[63].

In conclusion, PTK6 expression is associated with lymph node metastasis and poor survival in the IDC group but not in the SHT group of TNBC. Histological subtyping should be incorporated when evaluating PTK6 as a biomarker or therapeutic target. Prospective multi-center studies are warranted [38, 42, 61].

Abbreviations

Protein tyrosine kinase 6(PTK6),breast tumor kinase (BRK),Estrogen Receptor(ER), Progesterone receptor(PR), human epidermal growth factor receptor-2(HER2),Immunohistochemical (IHC),Fluorescence in situ hybridization (FISH), triple negative breast cancer (TNBC), Overall survival(OS), Disease-free survival(DFS),invasive ductal Carcinoma(IDC),special histological types carcinoma(SHT)

ER:Estrogen Receptor,PR:Progesterone receptor,HER2: human epidermal growth factor receptor-2,IHC: Immunohistochemical test,FISH: Fluorescence in situ hybridization,DFS:Disease-free survival,OS:overall survival,triple negative breast cancer :TNBC.

Declarations

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Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Research Committee of Nanchang People's Hospital(formerly The Third Hospital of Nanchang). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Competing interests

The authors declare no competing interests.

Consent for publication

Not applicable.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

Yuexia Chen and Xingmei Guo designed the research study, Suqing Cheng made contributions to the data gathering, Wei Qu and Jianhong Tu analyzed the data, Yuexia Chen wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approve the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors reviewed and approved the final version of the manuscript.

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Figures

Fig 1

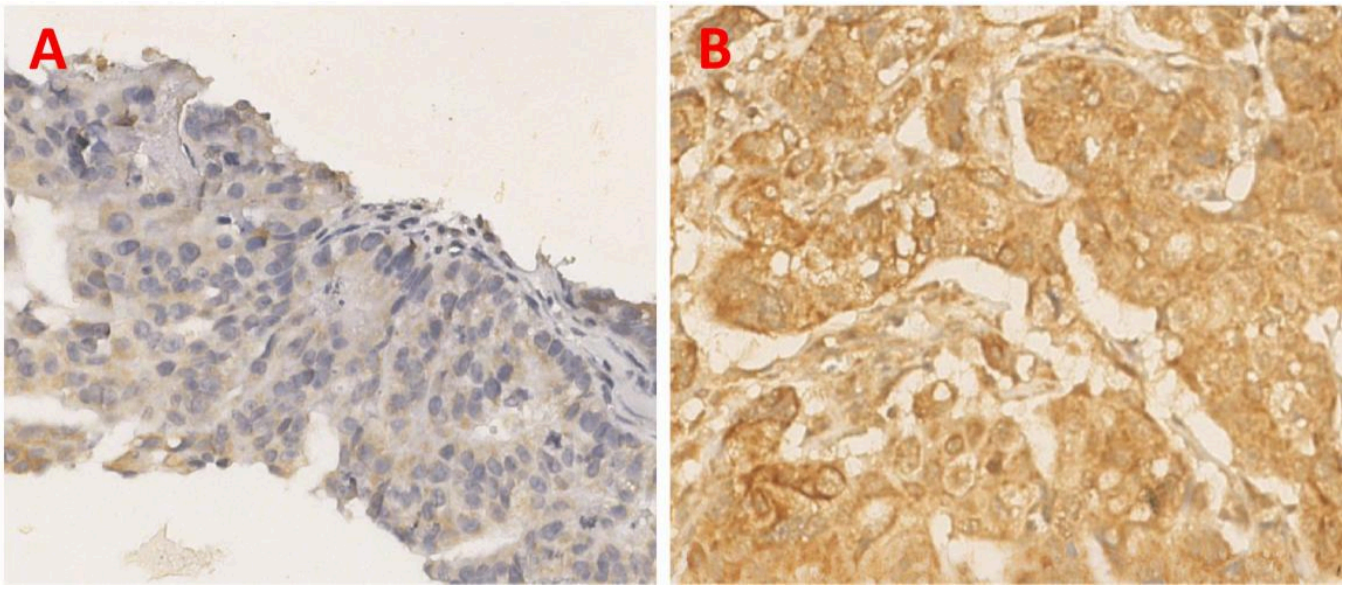


Figure 1

PTK6 staining (200X)

A.PTK6 negative;B.PTK6 positive

Fig 2

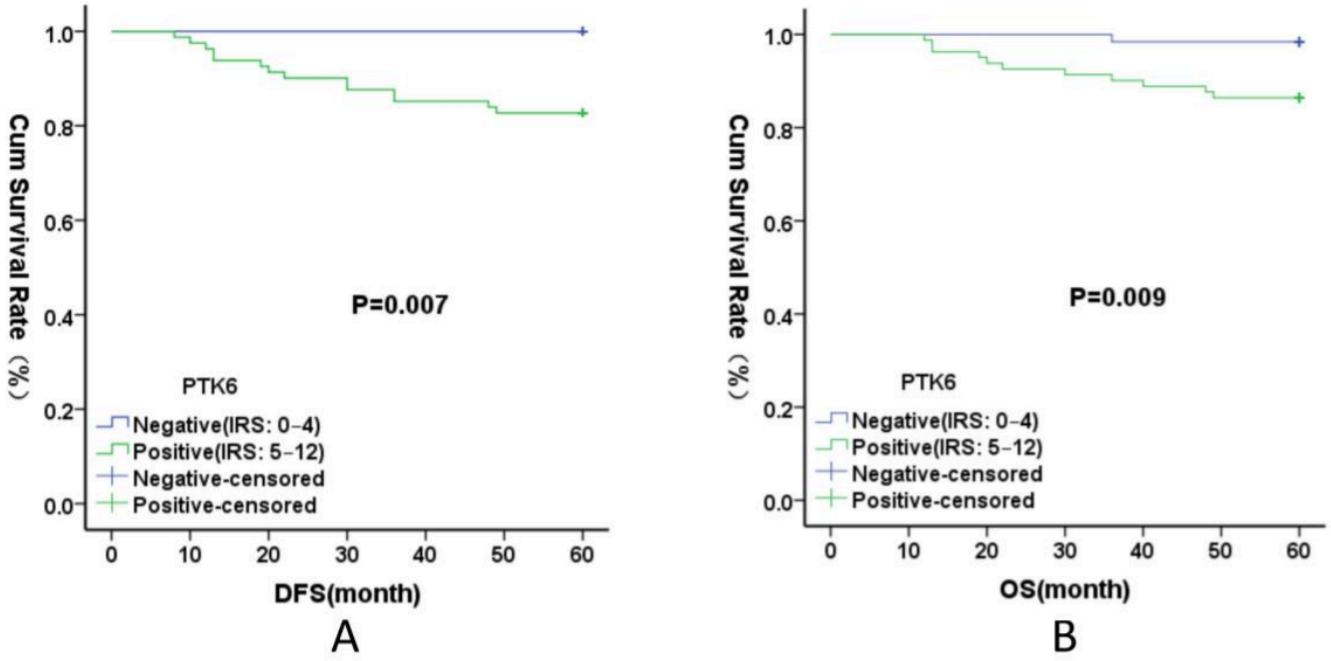


Figure 2

Kaplan-Meier analysis of DFS and OS in IDC group

A. DFS of IDC group; B. OS of IDC group

Fig 3

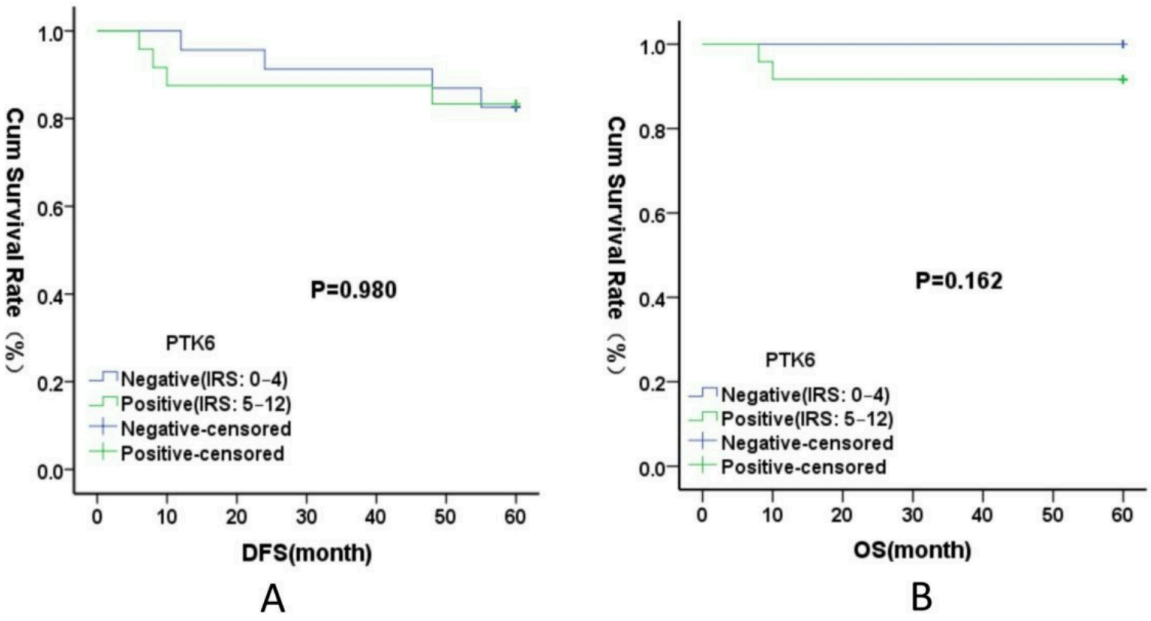


Figure 3

Kaplan-Meier analysis of DFS and OS in SHT group

A. DFS of SHT group; B. OS of SHT group