ACK1/TNK2 Tyrosine Kinase Overexpression Role in Gastric Cancer

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ABSTRACT

ACK1 or TNK2 integrate signals from ligand active receptor tyrosine kinases, which are exogenous oncogenic nonreceptor tyrosine kinases, to modulate intracellular signaling cascades 1. Several studies have revealed that ACK1/TNK2 is upregulated in various cancers and promotes tumor progression. This study aimed to determine the prognostic role TNK2 overexpression in gastric cancer. Presence of TNK2 overexpression in pathology blocks of patients with non-metastatic operated gastric cancer was used as prognostic marker and survival was investigated. The pathology samples of 129 patients were studied. Mean survival times (64.2 months) of the gastric cancer patients with TNK2 overexpression were significantly longer than those without overexpression (27.1 months) (p= 0.00). Median follow-up was 39 months. TNK2 overexpression rate was 75% in stage 1-2, 56% in stage 3 (p= 0.027). The positive effect of node negativity, ECOG performance status of 0-1 and TNK2 overexpression on survival in gastric cancer was also shown by multivariate analysis. We identified overexpression of the TNK2 in gastric cancer is correlated with good prognosis.

Keywords: TNK2, ACK1, Gastric cancer, Survival, Overexpression

ÖZET

ACK1/TNK2 Tirozin Kinaz Expresyonun Mide Kanserindeki Rolü

ACK1/TNK2, vücutta yaygın olarak exprese edilen onkojenik non reseptör tirozin kinazdır. Ligant ile aktive edilen reseptör tirozin kinazlardan gelen sinyalleri intraselüler sinyal kaskadını düzenlemek için bütünleştirir. Yapılan çalışmalarda ACK1/TNK2'nin birçok kanserde upregüle olduğu gösterilmiştir. Bu çalışmada gastrik kanserde TNK2 ekspresyonunun prognostik rolünü belirlemeyi amaçladık. TNK2 overexpresyonu prognostik belirteç olarak kullanıldı, opere olan lokal evre mide kanserinde sağkalıma etkisi araştırıldı. TNK2 overexpresyonu olan mide kanserli hastaların ortalama sağkalım süresi (64.2 ay) overexpresyon olmayanlara göre (27.1 ay) anlamlı olarak daha uzundu (p= 0.000). Medyan takip süresi 39 ay idi. TNK2 overexpresyonu evre 1-2'de %75, evre 3'de %56 oranında görüldü (p= 0.027). Mide kanserinde nod negatifliği, ECOG performans statusun 0-1 olması ve TNK2 overexpresyonun olmasının sağkalıma pozitif etkisi multivariate analiz ile de gösterildi. TNK2 overexpresyonu erken evrede daha fazla oranda görülür ve opere mide kanserinde iyi prognozla ilişkilidir.

Anahtar Kelimeler: TNK2, ACK1, Mide kanseri, Sağkalım, Overexpresyon

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INTRODUCTION

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related deaths worldwide.[2,3]

The signal transduction pathways that promote this rapid progress are not well understood. As a ubiquitously expressed non-receptor tyrosine kinase, ACK1/TNK2 has emerged as an essential transducer of a variety of extracellular signals.[4] ACK1/TNK2 gene amplification may cause ACK1 phosphorylation (p-ACK1), and activation of ACK1 signal transduction.[5,6] Activated ACK1 senses extracellular signals by interacting with activated receptor tyrosine kinases, such as AKT, EGFR, HER2 and MERTK.[6,7] TNK2 is primarily phosphorylated at Tyr284, enabling its kinase activation.[7,8] Several studies have shown the presence of ACK1/TNK2 amplification and overexpression in carcinogenesis of multiple tissue types such as lung, breast and prostate.[9-11] Overexpression level of ACK1/TNK2 correlates with tumor invasion and migration in renal and pancreatic cancers.[1,12]

ACK1/TNK2 gene is frequent in GC. However, little is known about the clinical roles and molecular mechanisms of ACK1 abnormalities in GC.[13] In this study, we aimed to evaluate the effect of TNK2 overexpression on survival and clinicopathologic features of non-metastatic gastric cancer and to present the results of patients in our center on this rare topic.

MATERIALS AND METHODS

In our study, we included pathology blocks of 129 non-metastatic gastric cancer diagnosed patients. Three-micron slices were prepared from paraffin blocks and deparaffinized in a 60^{0}C autoclave. Slides were labeled for assay and placed into the device (Leica Bond-Max, serial no: M21284, made in Melbourne Australia). Then, slides were placed into 5% hydrogen peroxide to block endogenous peroxide. We incubated the slides in liquid rabbit polyclonal antibody ACK1 (ABCAM, LOT: GR104132-4, 1/100; England); for 30 minutes. The post-primary antibody, the polymer solution and DAB mixtures (Leica Lot 11776) were applied for 10 minutes, respectively. We applied contrast staining with Mayer hematoxylin and protected the slides with covering material. We performed immunohistochemical staining. We used pulmonary carcinoma as a positive control.

We scored the immunohistochemical signals into four grades according to the intensity of staining as 0, 1+, 2+, and 3+. There were 46 0-stained patients (35.7%), 19 1+stained patients (14.7%), 31 2+stained patients (24%) and 33 3+stained patients (25.6%). We assumed immunohistochemical staining with +1 and above as TNK2 positive. TNK2 overexpression negative was present in 46 patients (35.7%) and positive in 83 patients (64.3%).

We used Kaplan-Meier and Cox-regression in the analysis of survival and Chi-Square test in the analysis of qualitative data.

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RESULTS

We examined pathology blocks of a total of 129 non-metastatic operated gastric cancer patients. TNK2 overexpression negative was present in

Table 1. Overall survival (OS) in patients with or without TNK2 expression

	Mean Survival Time (months)	95% CI		P
		Lower Bound	Upper Bound	
TNK2 overexpression (-)	27.1	20.5	33.8	0.000
TNK2 overexpression (+)	64.2	54.1	74.4	
Kaplan-Meier (Log-rank)				

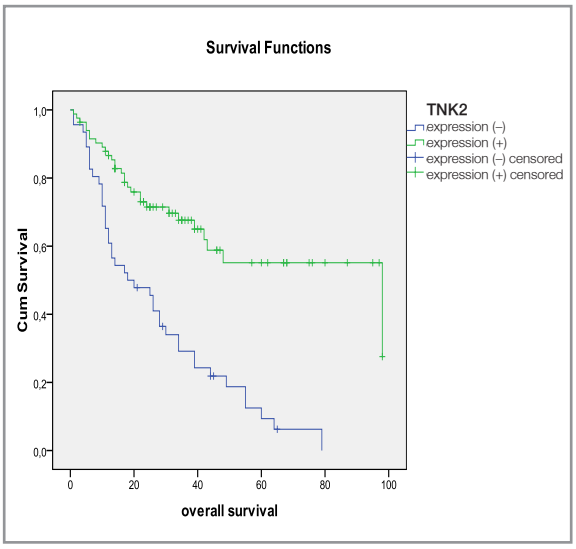

Figure 1. Prognostic value of TNK2 expression

46 patients (35.7%) and positive in 83 patients (64.3%). Forty-seven patients (36.4%) were female, 82 were male (63.6%). The mean age was 61 years. Median follow-up was 39 months. The mean survival time was significantly longer in patients with positive TNK2 overexpression (Table 1). We compared the clinicopathologic features of TNK2 overexpression positive and negative patients. The TNK2 overexpression positivity rate was 75% in stage 1-2, and 56% in stage 3 (p= 0.027) (Table 2) (Figure 1).

In the univariate analysis model, T stage, N stage, TNK2 overexpression, ECOG performance status (0-1/≥2), perineural invasion, vascular invasion, grade (I-II/III) have a significant effect on survival time in cancer (p< 0.05). We also demonstrated the effect of nodal status, the presence of TNK2 overexpression and ECOG performance status (0-1) on survival in gastric cancer by multivariate analysis (Table 3).

DISCUSSION

TNK2 is ubiquitously expressed in humans and senses extracellular signals by interacting with membrane-bound activated receptor tyrosine kinases such as EGFR, HER2, ALK, and MERTK.[14-17] The TNK2 gene is amplified in a variety of human carcinomas, including lung, ovarian, gastric and prostate cancers. TNK2 gene amplification causes the mRNA transcript level

Table 2. Potential predictors of prognosis in gastric cancer patient (n= 271)

	Univariate analysis 95% CI				Multivariate analysis 95% CI			
	HR	Lower bound	Upper bound	p	HR	Lower bound	Upper bound	p
Age (≤ 65/> 65)	1.36	0.83	2.19	0.215				
Stage (I-II/III)	2.83	1.67	4.78	**0.000**				
Grade (I-II/III)	1.50	0.92	2.45	0.100				
ECOG (0-1/≥ 2)	3.09	1.65	5.79	**0.000**	3.41	1.77	6.53	**0.031**
Gender (female/male)	1.03	0.63	1.68	0.897				
Surgical margin (negative/positive)	1.05	0.51	2.14	0.887				
pT stage (T1-T2/T3-T4)	4.06	1.27	12.93	**0.018**				
pN stage (N0/N1-N3)	3.63	1.89	6.95	**0.000**	3.40	1.77	6.53	**0.000**
TNK2 (overexpression -/+)	0.30	0.18	0.49	**0.000**	0.34	21.00	57.00	**0.000**
PNI (no/yes)	2.31	1.06	5.07	**0.000**				
VI (no/yes)	2.29	1.17	4.49	**0.016**				
Tumor size (≤ 5cm/> 5cm)	3.81	2.69	5.41	**0.000**				

Cox-Regression

Table 3. Correlations Between TNK2 Expression and Clinicopathologic Features in GC

		TNK2		
Factor	**Patient**	**Overexpression (–) (n= 46)**	**Overexpression (+) (n= 83)**	**p**
Gender				
Female	47	21 (44.7%)	26 (55.3%)	
Male	82	25 (30.5%)	57(69.5%)	0.105
Age				
≤65	76	28 (36.8%)	48 (63.2%)	
>65	53	18(34.0%)	35 (66.0%)	0.737
mean±SD	61.07±12.1	61.33±12.8	60.93±11.8	0.712
pT stage				
T1-2	14	3 (21.4%)	11 (78.6%)	
T3-4	115	43 (37.4%)	72 (62.6%)	0.239
pN stage				
N0	39	10 (25.6%)	29 (74.4%)	
N1-3	90	36 (40.0%)	54 (60.0%)	0.118
Grade				
I-II	52	18 (34.6%)	34 (65.4%)	
III	77	28 (36.4%)	49 (63.6%)	0.839
ECOG				
0-1	117	42 (35.9%)	75 (64.1%)	
≥2	12	4 (33.3%)	8 (66.7%)	0.860
Stage				
I-II	56	14 (25.0%)	42 (75.0%)	
III	73	32 (43.8%)	41 (56.2%)	**0.027**

to elevate.[10,18] Through these TNK2 activated signaling networks; TNK2 participates in cell survival, invasion, migration and tumorigenesis.[19]

In this study, the survival time was significantly longer in non-metastatic gastric cancer patients with TNK2 overexpression. We showed the effect of TNK2 overexpression on survival by multivariate analysis. Node negativity and ECOG performance status of ≤ 1 were independent good prognostic factors. TNK2 overexpression was more frequent in the early stage (stage I-II) patients than in advanced stage (stage III) patients. In our study, we found that TNK2 overexpression was not associated with prognostic factors such as T-stage and nodal involvement.

In the literature, there are few studies on the prognostic and predictive role of TNK2 in malignancies. In a study conducted in 2005 by Van der Horst et al., it was shown that in mice TNK2 overexpression increased metastasis and mortality.[10] In a study conducted in 2014, they compared 150 hepatocellular carcinomas operated patients with ACK1 low expression and high expression. They found that high expression was significantly associated with high grade, increasing number of tumors and increasing stage; and ACK1 was an independent prognostic and predictive marker.[20] In two other studies, TNK2 Tyr 284 phosphorylation kinase activation showed a negative correlation with survival in prostate and pancreatic cancer.[1,21]

There are two studies on gastric cancer related to this topic. The first study on stomach cancer was conducted in 2014 by Shinmura et al. In this study, TNK2 amplification was found to be an independent predictor factor, leading to an increase in malignant potential and poor prognosis in gastric

cancer.[6] TNK2 expression was also associated with advanced T stage and node involvement in this study. In another study of patients with gastric cancer, TNK2 was shown to be positively associated with lymph node metastasis and more advanced clinical stage.[13] Mean survival was 31.2 months in patients with ACK1 overexpression and 52.4 months in regular or low expression.

Despite existing evidence of TNK-2 being a poor prognostic feature among curatively operated gastric cancer patients, this study documented that TNK-2 overexpression is correlated with longer survival time. The conclusion might be attributable to the variations in patient populations: Patients enrolled may not be typical of the wider population. The other possible explanation for the difference, is the diversity in TNK-2 analysis techniques across the studies: The distinction in the secondary antibody of the immunohistochemical marker used, may have effected staining. In any case, our results by contrasting with the previous reports add some value to the literature given that there is paucity of data relevant to this issue, presenting necessity of validation in larger samples of participants.

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