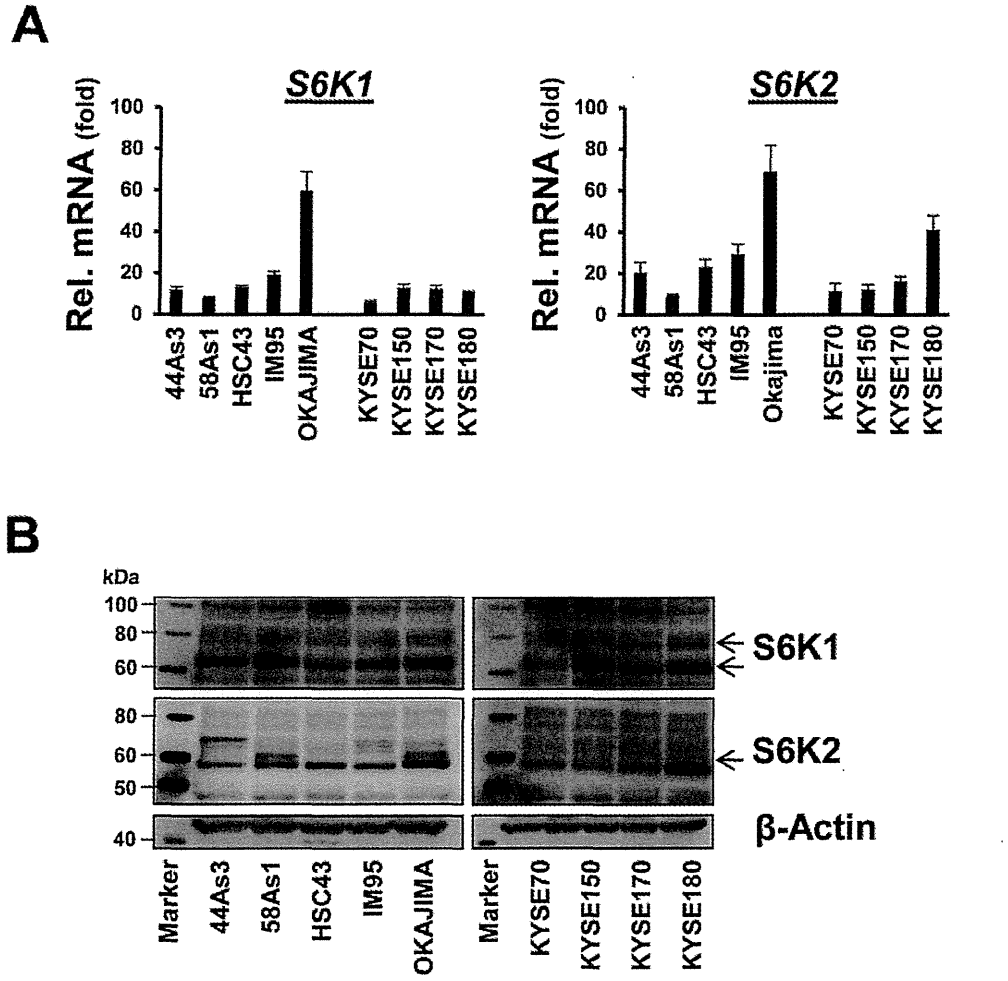

Figure 2. *S6 kinase-1 (S6K1) and S6K2 expression in cancer cell lines. A: The mRNA expression levels of S6K1 and S6K2 were determined using real-time RT-PCR. Rel. mRNA: Relative mRNA expression levels (target genes/GAPD ×10³). B: Western blot analysis for S6K1 and S6K2 protein. The arrows indicate the target proteins. β-actin was used as an internal control.*

percentages of the pathological stages were as follows: stage I, 8%; stage II, 12%, stage III, 29% and stage IV, 51%. Intestinal-type gastric cancer was observed in 42% (89/213) of the histologically-examined gastric carcinomas. The patient age, sex, pathological stage, and histology were not significantly associated with *S6K2*-amplification (Table I). The characteristics of the S6K2-amplified gastric carcinomas are summarized in Table II.

Finally, we examined the prognostic impact of *S6K2* amplification on OS after surgery. Although the sample size was relatively small (n=108), *S6K2* amplification was associated with a significantly shorter OS, compared with non-amplified cases, among patients with stage IV gastric cancer (log rank test, p=0.02; Figure 3C). Thus, *S6K2* amplification may be a novel prognostic factor for gastric cancer.

Discussion

Accumulating evidence demonstrates that the level of phosphorylation of S6K1 protein as detected using immunohistochemistry, which reflects activated mTORC1-S6K1 signaling, is increased in various types of cancer, including breast, lung, melanoma, hepatocellular carcinoma and glioma (3). Most of these studies showed that an increased phospho-S6K1 level was positively correlated with a poor prognosis, such as nodal metastasis and overall survival (3, 13-14). Therefore, the activation and overexpression of S6K1 enhances the malignant potential of the cancer cells. Regarding the gene amplification of *S6K1*, amplification is mostly observed in breast cancer, with a detection frequency of 6% to 14% using a Southern blot

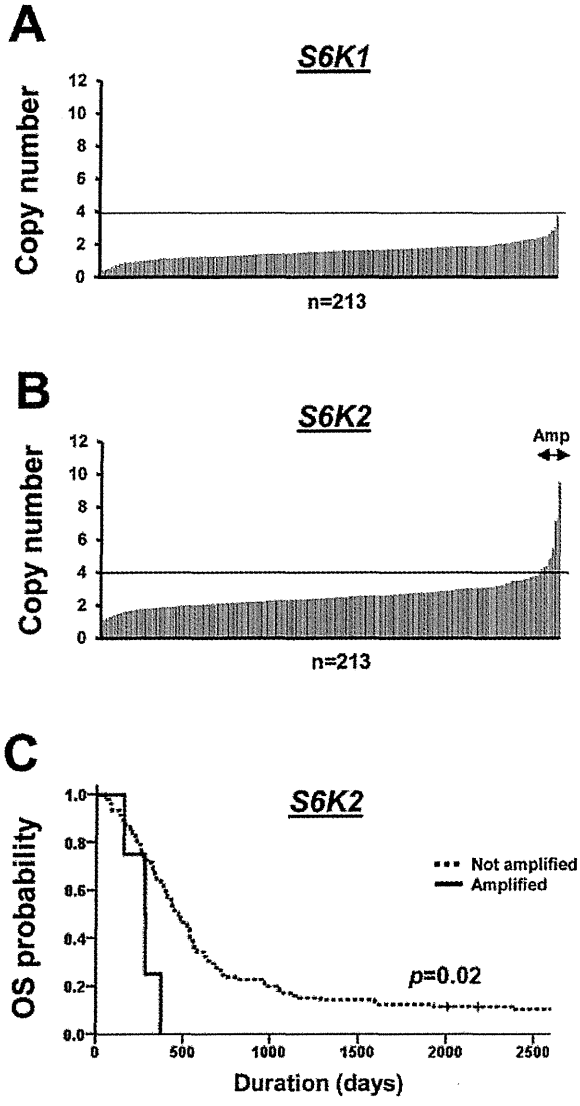

Figure 3. *Gene amplification of S6K1 and S6K2 in clinical gastric cancer samples. S6K1 (A) amplification and S6K2 (B) amplification were determined using the DNA copy number assay for 213 gastric cancer samples. S6K2 amplification over four copies was observed in 10 cases. C: Kaplan-Meier curves for overall survival (OS) in patients with stage IV gastric cancer. Patients were divided into two groups according to the S6K2 amplification. Amp, Gene amplification.*

Table I. *S6 kinase-2 (S6K2) amplification and clinicopathological features in gastric cancer.*

			S6K2 amplification		
		Total n=213	+ n=10	– n=203	*p*-Value
Age (years)	Range	31-91	45-75	31-91	0.94
	Median	63	63	63	
Gender	Male	147	7	140	0.78
	Female	66	3	63	
pStage	I	18	0	18	0.70*
	II	25	3	22	
	III	61	3	58	
	IV	108	4	104	
	Unknown	1	0	1	
Histology	Tub1	32	1	31	0.66**
	Tub2	44	1	43	
	Pap	5	0	5	
	Muc	8	1	7	
	Sig	11	2	9	
	Poor1	25	0	25	
	Poor2	88	5	83	

Tub, Tubular adenocarcinoma; Pap, papillary adenocarcinoma; Muc, mucinous adenocarcinoma; Sig, signet ring-cell carcinoma; Poor, poorly-differentiated adenocarcinoma; pStage, pathological stage. *Comparison between pStage I+II and III+IV. **Comparison between intestinal (Tub1, Tub2, Pap and Muc) and others. *p*-Values were calculated using the *t*-test for age and the χ^2 test for the other variables.

analysis, CGH, and fluorescence *in situ* hybridization (15-19). Thus, breast cancer is widely recognized to harbor a *S6K1* amplification in around 10% of samples. For other types of cancers, *S6K1* amplification was observed in three out of 16 (19%) medulloblastomas, in seven out of 38 (18%) diffuse large B-cell lymphoma combinations, and in two out of 17 (8%) ovarian cancer cell lines (20-22). In this study, we found that the 44As3 gastric cancer cell line harbored *S6K1* amplification, but amplification was not detected in clinical gastric cancer samples, suggesting that *S6K1* amplification is relatively rare in gastric cancer.

A limited number of studies have focused on the molecular and biological function of S6K2, despite the great number of studies that have examined S6K1 to date. Similarly, the dysregulation of S6K2 in cancer remains largely unknown. A recent study demonstrated that *S6K2* amplification was observed in nine out of 207 (4%) breast carcinomas, whereas the *S6K1* amplification was observed in 22 out of 206 (11%) (23). In addition, the *S6K2* amplification was correlated with a high mRNA expression level and was associated with a poor prognosis (23). Of note, another study from the same research group showed that the chromosomal region of 11q13, which includes the *S6K2* gene, was frequently co-amplified with the chromosomal region of 8p12, which includes another key downstream molecule of mTOR signaling, the eukaryotic translation initiation factor 4E binding protein-1 gene (24). Further study on such co-amplification is needed to understand the dysregulation of mTOR signaling. In addition, *S6K2* amplification may alter sensitivity to mTOR inhibitors, and thus further studies are warranted.

Table II. *Summary of patients with S6 kinase-2 (S6K2)-amplified gastric cancer.*

No.	Age	Gender	Macroscopic type*	Histology	pStage	OS (days)	S6K1 (CN)	S6K2 (CN)
1	45	M	2	Poor2	IIIa	394	1.5	4.1
2	64	M	4	Sig	IV	283	2.5	4.2
3	63	F	0-IIc	Sig	IIIb	4732+	1.8	4.3
4	52	M	2	Tub2	II	3935+	1.2	4.4
5	64	M	3	Tub1	II	1907	1.8	4.8
6	66	M	3	Poor2	IV	157	1.9	4.9
7	71	F	3	Poor2	II	835	1.3	5.5
8	75	M	3	Poor2	IV	280	2.0	7.1
9	73	M	4	Poor2	IV	373	1.7	7.3
10	57	F	3	Muc	IIIb	2742	1.6	9.5

No., Sample number; pStage, pathological stage; OS, overall survival; CN, copy number; *classification is based on the definitions of the Japanese Research Society for Gastric Cancer; + for OS, indicates the patient was still alive at the time of writing. Tub, Tubular adenocarcinoma; Muc, mucinous adenocarcinoma; Sig, signet ring-cell carcinoma; Poor, poorly-differentiated adenocarcinoma; pStage, pathological stage.

In conclusion, we found that the *S6K2* amplification was observed in gastric cancer at a frequency of 4.7%, and its amplification was related to a poor outcome. Our results may provide an insight into the dysregulation of mTOR signaling in gastric cancer.

Conflicts of Interest

None.

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