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Is NEDD4-1 a negative regulator of phosphatase and tensin homolog in gastric carcinogenesis?

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Abstract

The expression of phosphatase and tensin homolog (*PTEN*), a tumor suppressor gene, is frequently down-regulated in gastric carcinomas due to mutation, loss of heterozygosity, and promoter hypermethylation. However, it is unknown if additional mechanisms may account for the down-regulation of PTEN expression. While neuronal precursor cell-expressed developmentally down-regulated 4-1 (NEDD4-1) is believed to be a potential dual regulator of PTEN, there are conflicting reports regarding their interaction. To gain further insight into the role of NEDD4-1 and its association with PTEN in gastric carcinoma development, we measured the protein expression of NEDD4-1 and PTEN in gastric mucosae with various pathological lesions and found that NEDD4-1 increased from normal gastric mucosa to intestinal metaplasia and decreased from dysplasia to gastric carcinoma. These changes did not correlate with PTEN expression changes during gastric carcinogenesis. Moreover, we found similar results in protein levels in the primary tumors and adjacent non-tumorous tissues. These results differ from a previous report showing that expression of NEDD4-1 is up-regulated in gastric carcinomas, and show a more complex pattern of NEDD4-1 gene expression during gastric carcinogenesis.

Key words: Neuronal precursor cell-expressed developmentally down-regulated 4-1; Phosphatase and tensin homolog; Gastric carcinogenesis; Immunohistochemistry

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TO THE EDITOR

One of the aims of cancer research is to identify mechanisms controlling the loss of tumor suppressors that promote cancer development. Expression of phosphatase and tensin homolog (*PTEN*), a tumor suppressor gene[1-3], is frequently down-regulated in gastric carcinomas (GC) due to mutation, loss of heterozygosity, and promoter hypermethylation[4-8]. In an effort to determine if neuronal precursor cell-expressed developmentally down-regulated 4-1 (NEDD4-1), a potential dual regulator of PTEN[9,10], plays a role in the development of gastric carcinoma, Kim *et al*[11] investigated NEDD4-1 protein expression in 60 specimens of gastric carcinoma tissues using immunohistochemistry. They found that 75% of GC were immunopositive for NEDD4-1, whereas the normal gastric mucosal cells displayed a weak or

Table 1 Differential expression of neuronal precursor cell-expressed developmentally down-regulated 4-1 and phosphatase and tensin homolog at different histological gastric tissue specimens

Group	*n*	Age (yr)	Sex (M/F)	NEDD4-1							PTEN						
				-	+	++	+++	Mean rank	Group	*P* value	-	+	++	+++	Mean rank	Group	*P* value
Normal gastric mucosa	21	50.0 ± 14.1	13/8	2	6	11	2	87.64	1 *vs* 2	0.567	3	10	4	4	108.26	1 *vs* 2	0.607
									1 *vs* 3	0.127						1 *vs* 3	0.211
									1 *vs* 4	0.519						1 *vs* 4	0.448
									1 *vs* 5	0.225						1 *vs* 5	0.001
									2 *vs* 3	0.399						2 *vs* 3	0.062
Chronic gastritis	21	54.2 ± 14.7	10/11	0	8	9	4	96.26	2 *vs* 4	0.983	1	10	8	2	116.21	2 *vs* 4,	0.185
Intestinal metaplasia	41	54.5 ± 12.4	21/20	3	6	23	9	106.16	2 *vs* 5	0.059	13	15	9	4	91.55	2 *vs* 5	0.000
Dysplasia	48	57.4 ± 12.5	26/22	4	14	19	11	95.83	3 *vs* 4	0.364	13	16	15	4	98.08	3 *vs* 4	0.535
Gastric carcinoma	50	54.0 ± 12.3	31/19	7	21	18	4	73.13	3 *vs* 5	0.001	30	10	9	1	65.9	3 *vs* 5	0.012
Overall	181	54.6 ± 13.8	101/80	16	55	80	30		4 *vs* 5	0.029	60	61	45	15		4 *vs* 5	0.001

NEDD4-1: Neuronal precursor cell-expressed developmentally down-regulated 4-1; PTEN: Phosphatase and tensin homolog.

no immunoreactivity. NEDD4-1, an E3 ubiquitin-protein ligase, is believed to play two opposite roles in regulation of PTEN[9,10]. On one hand, it exercises an oncogenic function by catalyzing PTEN polyubiquitination, resulting in degradation of PTEN protein[9]. On the other hand, it exerts a tumor suppressive function by catalyzing PTEN monoubiquitination and regulating PTEN nuclear transport[10]. Together, these data suggest that NEDD4-1 may play a role in gastric carcinoma development and that it may function as a negative regulator of PTEN.

To gain further insight into the role of NEDD4-1, and more importantly, its association with PTEN in gastric carcinoma development, we measured expression of NEDD4-1 and PTEN by immunohistochemistry in biopsies or surgical specimens of 181 patients with various developmental stages of gastric carcinoma collected from January 2007 to September 2009 at the First Affiliated Hospital of Nanchang University. The specimens included 21 cases of normal gastric mucosa (NGM), 21 cases of chronic gastritis (CG), 41 cases of intestinal metaplasia (IM), 48 cases of dysplasia (Dys), and 50 cases of GC (Table 1). There were no significant differences in the age or gender distributions among these groups. These tissue specimens were sectioned and immunostained using the PV-9000 Polymer Detection System (Zhongshan Goldenbridge, Beijing, China), then reviewed and scored semi-quantitatively, as described previously[12]. An additional 15 pairs of primary tumors and adjacent non-tumorous tissues were subjected to total cellular protein isolation and Western blot analysis of NEDD4-1 and PTEN expression. This work was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University. Primary antibodies used in this study were: rabbit polyclonal anti-human NEDD4-1 (Millipore, Billerica, MA, United States), PTEN (Abcam, Cambridge, United Kingdom), and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, United States).

The expression of NEDD4-1 was found to increase from NGM to IM, but to decrease from Dys to GC ($P < 0.05$). NEDD4-1 expression level was significantly decreased in GC compared with IM or Dys (Figure 1 and Table 1, $P < 0.05$). There were no significant differences in NEDD4-1 expression between GC and NGM or CG (Figure 1 and Table 1). The expression of PTEN was found to increase from NGM to CG, but to decrease from IM to GC ($P < 0.05$). The PTEN expression level was significantly decreased in GC compared with NGM, CG, IM and Dys (Figure 1 and Table 1, $P < 0.05$). Analysis of the data from cases of GC did not show an obvious association between NEDD4-1 or PTEN expression levels and clinicopathological grades. In addition, we did not find any correlations between NEDD4-1 expression and PTEN expression in different stages. In primary GC, 13 (86.7%) of 15 cases showed a reduction in PTEN expression in tumor tissues compared with the corresponding non-tumorous mucosa. The mean level of PTEN expression was significantly lower in tumor samples than in the non-cancerous counterparts (Figure 2, $P < 0.05$). However, there was no significant difference in the expression of NEDD4-1 between tumor samples and the non-cancerous counterparts (Figure 2, $P < 0.05$).

Since Kim *et al*[11] showed an increased expression of NEDD4-1 in GC, we expected to detect increased NEDD4-1 expression in GC. However, we found that NEDD4-1 was increased in the early stages of gastric carcinogenesis, but was then decreased in GC. In addition, we did not find increased NEDD4-1 expression in tumor tissues compared with the corresponding non-tumorous mucosa. More importantly, there was no correlation between NEDD4-1 expression and PTEN expression.

There are several factors that may explain the inconsistencies between the results from our study and those from others. First, although NEDD4-1 is believed to be a potential dual regulator of PTEN, there are discrepancies regarding the nature of the relationship between NEDD4-1 and PTEN. Fouladkou *et al* reported that NEDD4-1 is not the E3 ligase regulating PTEN stability and subcellular localization[9,10,13]. We did not see any correlation between NEDD4-1 and PTEN expressions, suggesting that NEDD4-1 may not be a regulator of PTEN in gastric carcinogenesis. Second, there was dif-

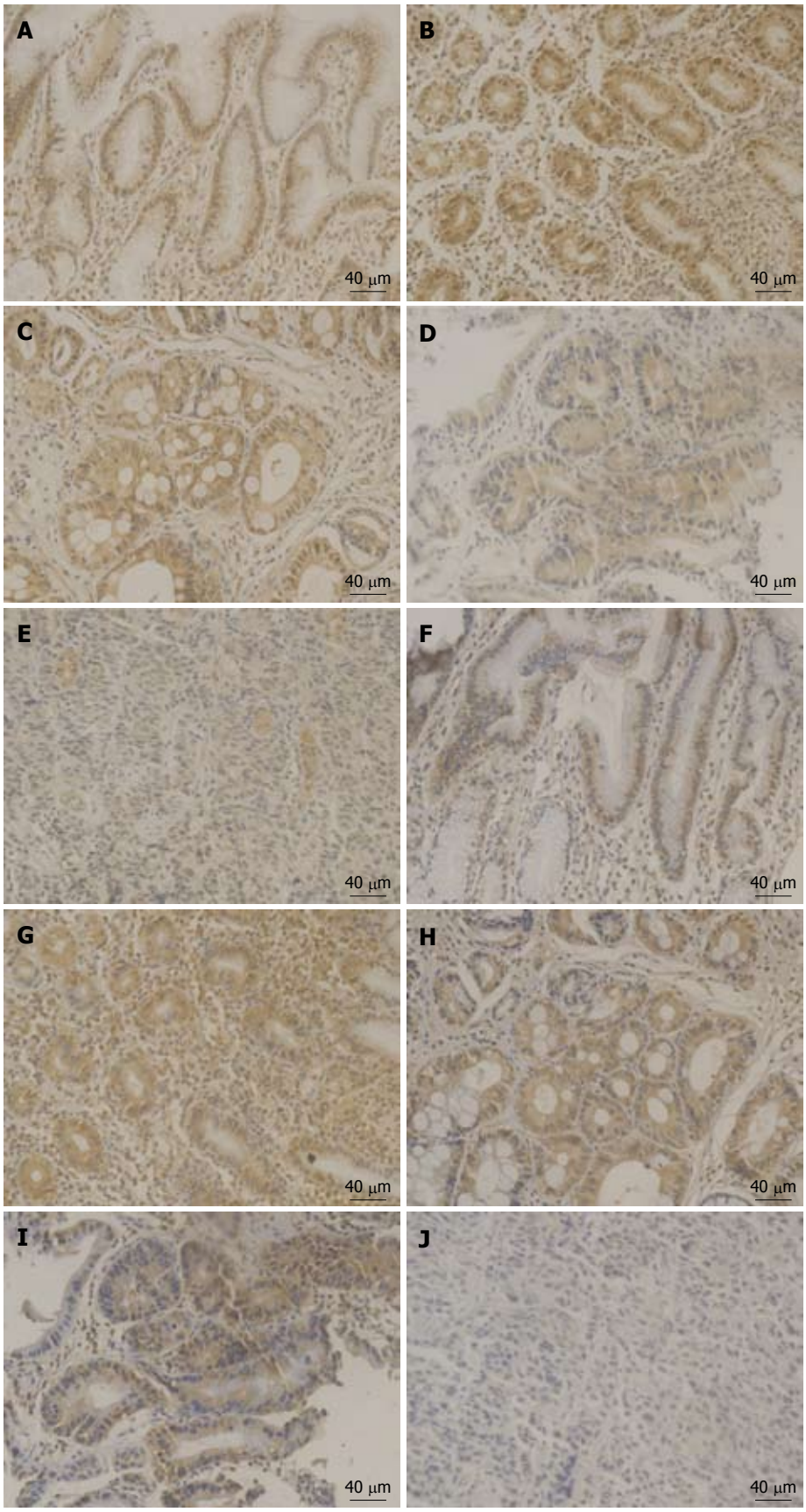

Figure 1 Immunohistochemical staining of neuronal precursor cell-expressed developmentally down-regulated 4-1 and phosphatase and tensin homolog protein in different histological samples of gastric tissues. A: The expression of neuronal precursor cell-expressed developmentally down-regulated 4-1 (NEDD4-1) in normal gastric mucosa (NGM); B: The expression of NEDD4-1 in chronic gastritis (CG); C: The expression of NEDD4-1 in intestinal metaplasia (IM); D: The expression of NEDD4-1 in dysplasia (Dys); E: The expression of NEDD4-1 in gastric carcinoma (GC); F: The expression of phosphatase and tensin homolog (PTEN) in NGM; G: The expression of PTEN in CG; H: The expression of PTEN in IM; I: The expression of PTEN in Dys; J: The expression of PTEN in GC. Magnification: × 200.

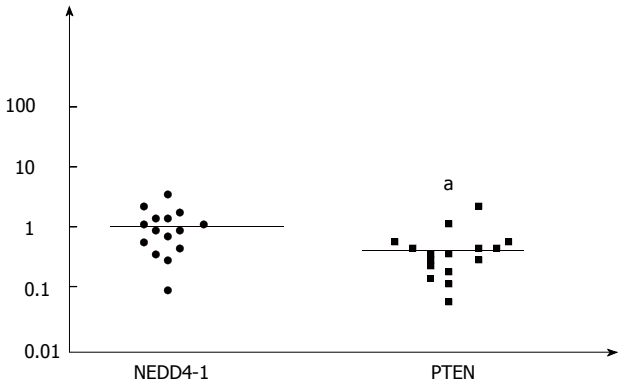

Figure 2 Relative neuronal precursor cell-expressed developmentally down-regulated 4-1 and phosphatase and tensin homolog protein expression level in gastric carcinoma compared to adjacent non-tumorous mucosa. Immunoblots of neuronal precursor cell-expressed developmentally down-regulated 4-1 (NEDD4-1), phosphatase and tensin homolog (PTEN) were scanned and the relative protein expression level of tumor samples vs their non-cancerous counterparts is expressed as % of β-actin. $^{a}P < 0.05$.

ference in the genetic background of the cohort from our study and a previous study. In our study, patients were from southern China, whereas in the study by Kim *et al*[11], patients were from South Korea. In addition, the scoring systems of the two studies were different. In conclusion, we show in the present study that NEDD4-1 and PTEN are distinctly expressed in the various stages of gastric carcinogenesis. In addition, PTEN expression was significantly lower in primary tumors than in adjacent non-tumorous tissues, while there was no difference in NEDD4-1 expression between primary tumors and adjacent non-tumorous tissues. Therefore, NEDD4-1 may not be a regulator of PTEN in gastric carcinogenesis. To elucidate the roles of NEDD4-1 in gastric carcinogenesis, especially the role in regulation of PTEN function, further studies with larger samples in the various stages of gastric carcinoma are needed.

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