DLG7/DLGAP5 as a potential therapeutic target in gastric cancer

Kang Li[1], Xiangsheng Fu[2], Ping Wu[3], Bianba Zhaxi[1], Hanhuan Luo[1], Qijie Li[4]

[1]Department of Gastroenterology, People's Hospital of Tibet Autonomous Region, Lhasa, Tibet 850000, China;
[2]Department of Gastroenterology, First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610041, China;
[3]Department of Occupational Diseases, The Second Affiliated Hospital of Chengdu Medical College Nuclear Industry 416 Hospital, Chengdu, Sichuan 610051, China;
[4]Department of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China.

To the Editor: Gastric cancer is a highly invasive disease. An estimated 1.09 million cases were diagnosed and 770,000 gastric cancer-related deaths occurred only in 2020.[1] Advanced gastric cancer can no longer be subjected to surgery and can only be treated with sequential lines of chemotherapy. However, the survival period is usually short.[2] Therefore, it is of utmost importance to identify new and more effective treatments and therapeutic targets to combat gastric cancer. Disc large homolog 7 (DLG7) is the microtubule-associated protein encoded by the DLG associated protein 5 (*DLGAP5*) gene, which affects chromosome rearrangement and gene stability[3]; thus, its overexpression may cause canceration. Some studies revealed that DLGAP5 is highly expressed in gastric cancer tissues, suggesting that it may also have a role in this disease. However, the role and mechanism of DLGAP5 in gastric cancer are still unclear.[4] Therefore, this study aimed at exploring the role of DLGAP5 in the growth and invasion of gastric cancer as well as the potential use as a therapeutic target to combat this disease.

This study was reviewed and approved by the Ethical Committee of People's Hospital of Tibet Autonomous Region, China (approval number: ME-TBHP-20-KJ-021), and the informed consent was obtained from all participants. The six pairs of gastric cancer tissues and adjacent normal tissues were collected from the patients who had been admitted to the People's Hospital of Tibet Autonomous Region for surgical treatment. The expression of both DLGAP5 mRNA and DLG7 protein in gastric cancer tissues was significantly higher than that in the normal tissues adjacent to cancer ($P < 0.050$) [Figure 1A], confirming our previous results on the whole genome expression profiling. In addition, Western blot results showed that DLG7 protein levels in gastric cancer cell lines MKN-45 (hereinafter referred to as MKN), BGC-823, and MGC-803 (hereinafter referred to as MGC), not in AGS, were higher than that in normal gastric epithelial cells GES1 ($P < 0.010$) [Figure 1B]. Liu *et al*[4] analyzed the data obtained from the cancer genome map and gene expression comprehensive database and found the presence of a selective upregulation of DLGAP4 and DLGAP5 gene expression in gastric cancer tissues in comparison with the normal gastric tissues, but they only demonstrated the critical role of DLGAP4 in gastric cancer without investigating DLGAP5. Thus, the specific function of DLGAP5 in the development and progression of gastric cancer has not been reported. Further in this study, DLGAP5 shRNA (shDLGAP5) was transferred into MKN and MGC cells by lentivirus infection that was used to investigate the possible function of DLGAP5 in gastric cancer.

The Cell Counting Kit-8 (CCK-8) assay demonstrated that the proliferation ability (as evaluated by the optical density [OD] value) was significantly reduced in DLGAP5 knockdown MKN and MGC cells compared with the normal cells at 48 hours, 72 hours, and 96 hours ($P < 0.010$), whereas the proliferation ability in the negative control group was scarcely modified, suggesting that the overexpression of DLGAP5 might promote the proliferation of gastric cancer cells [Figure 1C]. Tsou et al[5] demonstrated that DLGAP5 overexpression enhances the proliferation ability of human cells. This study confirmed that the down-regulation of DLGAP5 expression exerted a negative effect on the proliferation of gastric cancer cells. DLG7 is involved in the stabilization of the oncoprotein Gankyrin, which plays a role in the ubiquitination and degradation of p53.[6] The overexpression of DLGAP5 led to the accumulation of

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Kang Li and Xiangsheng Fu contributed equally to the work.

Correspondence to: Dr. Qijie Li, Department of Anesthesiology, Laboratory of Anesthesia and Critical Care Medicine, Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China
E-Mail: qjlbsh2019@163.com

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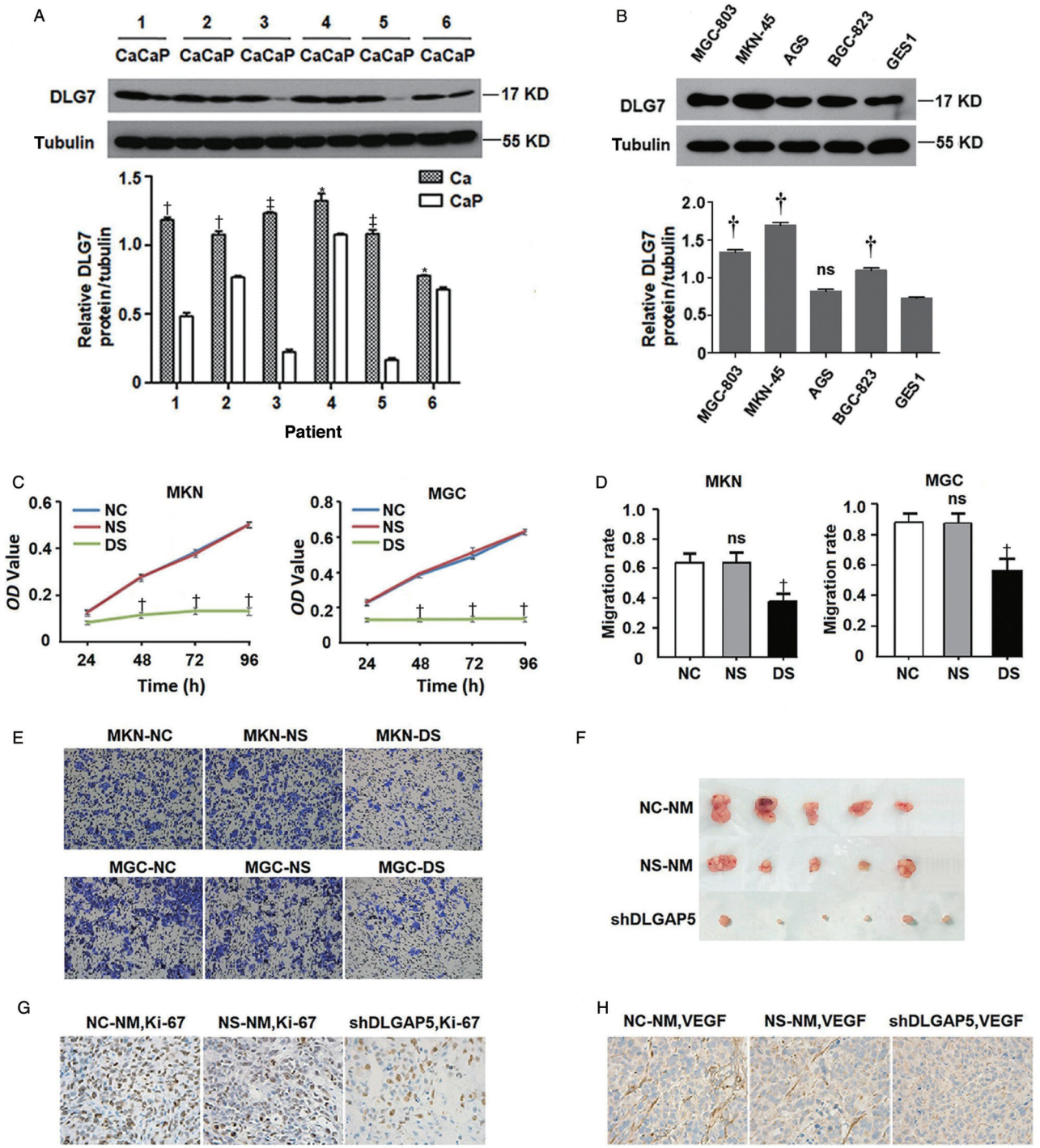

Figure 1: (A) DLG7 protein levels in six pairs of gastric cancer tissues and normal tissues adjacent to cancer as detected by Western blot. Tubulin was used as the loading control, and the protein bands were quantified and compared with the CaP group (Ca: Gastric cancer tissues; CaP: Adjacent gastric cancer tissues), $^{*}P < 0.050$, $^{\dagger}P < 0.010$, $^{\ddagger}P < 0.001$, $n = 6$. (B) DLG7 protein levels in the cell lines AGS, BGC-823, MGC-803, MKN-45, and GES1 as detected by Western blot. The protein bands were quantified and compared with the normal gastric epithelial cell line GES1, $^{\dagger}P < 0.010$; ns, no statistical significance, $n = 3$. The proliferation (C) was detected by CCK-8 assay, the migration ability (D) was evaluated by the wound-healing assay, and the invasion ability (E) was evaluated by the crystal violet dyeing (original magnification ×400) of Transwell invasion test in MKN and MGC cells after DLGAP5 gene knockdown, compared with the NC group, $^{\dagger}P < 0.010$; ns, no statistical significance, $n = 3$ (NC: Normal control group; NS: shRNA virus infection negative group; DS: DLGAP5 shRNA virus infection group). (F) Tumor formation from MGC cells in nude mice in each group, (G) Ki-67 protein expression in the tumor of nude mice in each group as detected by immunohistochemistry (original magnification ×400), (H) VEGF protein expression in the tumor of nude mice in each group as determined by immunohistochemistry (original magnification ×400) (NC-NM: Normal MGC cells were inoculated in nude mice, $n = 5$; NS-NM: MGC cells infected with viruses containing negative shRNA were inoculated in nude mice, $n = 5$; shDLGAP5: viruses infected with DLGAP5 shRNA were inoculated in nude mice, $n = 6$). DLG7: Disc large homolog 7; DLGAP5: DLG associated protein 5; NS-NM: Negative shRNA; OD: Optical density; shDLGAP5: DLGAP5 shRNA; VEGF: Vascular endothelial growth factor.

Gankyrin, and thus resulting in the degradation of p53 mediated by ubiquitin, which may explain the effect of DLG7/DLGAP5 on the proliferation of gastric cancer cells.[7]

The results of the scratch assay revealed that the migration ability of gastric cancer cells was significantly decreased after DLGAP5 gene knockdown in contrast with the migration of normal MKN and MGC cells ($P < 0.050$), whereas the change was not significant between the normal cells and the negative control group [Figure 1D]. Transwell invasion experiments showed that the invasion of MKN and MGC cells was significantly inhibited after DLGAP5 gene knockdown [Figure 1E]. These results suggested that the knockdown of the DLGAP5 gene might inhibit the migration and invasion ability of gastric cancer cells. The study of Wang *et al*[8] showed that DLGAP5 enhanced the invasiveness and migration of cancer cells in non-small cell lung cancer, and a similar phenomenon was also discovered in gastric cancer in this study.

Normal MGC cells (NC-NM), MGC cells infected with the virus containing the shDLGAP5 or negative shRNA (NS- NM), were used to establish a nude mouse tumor model so as to further verify the function of DLGAP5 in the formation and development of gastric cancer. The results showed that the tumor size of nude mice in the shDLGAP5 group was significantly lower than that in the NC-NM group, but no significant change was observed in the NS- NM group regarding these parameters [Figure 1F]. Moreover, the immunohistochemistry results showed that K-67 protein expression in the shDLGAP5 group was significantly reduced [Figure 1G]. These results suggested that DLGAP5 gene knockdown could significantly inhibit the growth of gastric cancer cells *in vivo*. In addition, hematoxylin and eosin staining results revealed that the knockdown of the DLGAP5 gene led to a significant decrease in the tumor blood vessels and a decrease in the ability of angiogenesis in nude mice compared with the NC-NM group. Finally, the immunohistochemistry results demonstrated that vascular endothelial growth factor (VEGF) expression in the shDLGAP5 group was significantly decreased compared with its expression in the NC-NM group [Figure 1H]. These results suggested that DLGAP5 gene knockdown could inhibit the angiogenesis in gastric cancer *in vivo*.

In conclusion, the ability of growth, metastasis, and invasiveness of gastric cancer cells after DLGAP5 gene knockdown was significantly reduced, and knockdown of DLGAP5 inhibited the growth in gastric cancer cells and tumor angiogenesis in nude mice, which demonstrated that DLG7/DLGAP5 promoted the development and progression of gastric cancer, thus, might be considered as a target in the treatment of gastric cancer.

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Conflicts of interest

None.

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